

23 October 1980

How to change the Nobel Prize rules

It is never the right time to raise questions about the institution of the Nobel Prize. For much of the year, most people are uninterested. The Nobel committees, of course, beaver away throughout the year and it is just possible that there are some in the world outside who, after a hard day in the laboratory, allow themselves to drift to sleep wondering whether what they have just done will one day produce a telegram from Stockholm. For most people, however, the phenomenon of the Nobel Prize is a seasonal phenomenon — perhaps fittingly something to do with the run-up to Christmas. And this, of course, is the season when the telegrams go out.

The danger of raising questions about the institution of the Nobel Prize at this stage is that the entirely proper pleasure of the recipients may be undermined. It is therefore right and proper that last week's prizewinners and their friends should know that their awards have generated just as much pleasure, and deserve congratulation as wholehearted, this year as in the past. Indeed, last week's award to Dr Frederick Sanger should prove that it is possible to distinguish between the merits of the prizewinners and the merits of the scheme. There can be few who are not delighted that such a modest man, yet such an able and imaginative scientist, should have been awarded his second prize.

So what can be wrong with an institution capable of such enlightened decisions? Is there not inherent virtue in an organization whose doings stimulate the pursuit of excellence? Why should there be anxieties about the Nobel Foundation when, by the careful management of its endowment and the diligent pursuit of its procedures, it has become a conspicuous adornment of the scientific profession? And if the institution of the Nobel Prize has stimulated later benefactors to found charities whose work consists of the award of prizes in other fields, from mathematics to immunology, is that not a sign that the market for scientific prizes is buoyant — benefactors seem to like endowing prize funds and scientists to enjoy receiving prizes? There is force in all these implicit arguments. The doubts are harder to define.

One complication is that of envy. Especially in these egalitarian days, there are constant grumbles that the singling out of individuals, however distinguished, for special honour is offensive. Discovery, the argument goes, is often a matter of luck — of being in the right laboratory at the right time. And if it turns out that a person makes an outstanding contribution to the development of his field, might that not merely reflect the value of the groundwork done by his predecessors? After all, it was no less a person than Newton who confessed that "if I have seen further than others, it is because I have stood on the shoulders of giants".

This line of argument is sadly inappropriate, even discreditable. Nobody these days will deny the importance of the climate in which research is carried out in fostering discovery. Teamwork is important. So too is the way in which people brought together, apparently often by accident, in productive laboratories derive extra benefit from being there rather than in some relatively backward place. From the generality of the scientific profession (as of any other creative profession), some able men and women are distinguished by their creativity, clarity of mind and sheer drive. It is proper that these qualities should be recognized for what they are — disproportionately powerful stimulants of discovery. Prizes are one way of recognizing such outstanding qualities, and should be valued.

Such doubts as there are about the prize-giving system are thus largely second-order effects. The Nobel Prizes are most often discussed because they are known widely outside the profession

and because they are commonly supposed to deal with the whole of science under the three rubrics of physics, chemistry and "medicine and physiology". (Peace, literature and economics are dealt with separately.) It is therefore important that the scope of the Nobel system is by no means as catholic as it is usually supposed to be. The gap left by the exclusion of mathematics from the terms of Alfred Nobel's will has now been more than amply filled by the annual Field Prize, but it remains an anomaly bordering on the nonsensical that, for example, David Hilbert would not have been awarded a prize under the present rules. It is stranger still that growing fields such as astronomy and the earth sciences should on the face of things be excluded. Although the appropriate committees have in the past few years found ways of counting as physicists Vine and Matthews (because they measured magnetic anomalies on the sea floor) and a number of radio-astronomers (no doubt because radioastronomy sprang from physics laboratories), it is hard to see the Nobel Foundation screwing up its courage to award a prize to, say, Hubble (the astronomer) or Napier (the meteorologist). Whether Darwin would have qualified is an open question.

On the face of things, these complaints are not complaints against Nobel Prizes as such but at the scope of the present system. There is more than that to be said, however. When Alfred Nobel wrote his will at the end of the nineteenth century, he and his advisers could not have known how the balance of science would shift in the succeeding years. They set out to define a set of prizes covering the whole of the science they knew. Their intentions have been overtaken by events. The result is that some fields of science are well covered and others only marginally. The consequences are potentially harmful for the reputation of the prizes which are awarded, if only because of the sense of fickleness that is engendered. By all accounts, the Nobel Foundation is aware of this problem but insists that it would be impossible to amend the terms of Nobel's will. Can that be so? Other charitable foundations are constantly having to change the terms of their trust deeds by applications to the courts so as to fulfil their founders' wishes more adequately. Why should the Nobel Foundation be prevented from following suit?

Modest reform along these lines would help make the Nobel Prize system seem more equitable, taking one field of science with another, but this is not the only respect in which the fairness of the system has been called into question. In the past few years, there have been several occasions when only one member of a small team responsible for some important development has been awarded a prize. This practice appears especially unfair when the team consists only of two people and the prize is awarded to the more senior. Some years ago, there was something of a public controversy in Britain about the award of a prize to Dr Anthony Hewish for the discovery of pulsating stars when his graduate student, Miss (as she then was) Jocelyn Bell went unsung. Both the principals came out of the affair with credit, as no doubt would emerge from any new controversy Dr W. Gilbert (one of this year's winners) and Dr A.M. Maxam (whose name is linked with that of Dr Gilbert in the scientific literature for their development of their novel nucleotide sequencing technique, but who has not this year been given a prize). Like judges in a beauty contest, the Nobel committee brings disappointment as well as pleasure. Strict fairness is unattainable, but there is a sense in which the growth of the practice of close collaboration between groups of scientists may be a threat to the credibility of the system.

A still more serious danger is that the Nobel committees will



from time to time let their pent-up sense of mischief get the better of them. In other fields, and in the award of the literature prize in particular, they are prone to let rather temporal considerations get the upper hand. An award to a distinguished writer seems the more delicious (from Stockholm) if the result should be problems with an exit visa from, say, the Soviet Union. There could be just an element of that tail-twisting in this year's prize (shared with Sanger and Gilbert) to Dr Paul Berg of Stanford University, who is, of course, a molecular biologist of the highest standing and the first to join two pieces of DNA together synthetically and also a distinguished teacher. But Dr Berg has also been, perhaps unwillingly, one of the advocates of the regulation of genetic manipulation. Nobody will begrudge Dr Berg his prize, and nobody in his senses will deny that the progress of science is effected not merely by startling discoveries but also by the genial influence of men and women who help to ensure that a new subject is widely known, properly understood and regarded with

enthusiasm. This, for what is worth, was the late J. Robert Oppenheimer's influence in physics in the 1930s, but he died without a Nobel Prize. Nobel's will may be intact, but have the rules been subtly changed?

That such questions are asked of the Nobel Foundation is a measure of its success. For better or worse, the Nobel Prize has come to stay. The need now is to make sure that it creates more pleasure than grumbling disappointment. Broadening the terms of reference of the will would help. A more explicit statement of the reasons for making an award, and a more explicit recognition by the Nobel committees of the teamwork involved in novel discoveries, would often help to disembarass those who are awarded prizes and who are frequently heard to say "It's not for me but for my lab". Indeed, there is even a case for thinking that the Nobel Foundation might itself give expression to that sentiment by making the award, as at present, to the distinguished scientist but sending the cheque to his institution.

Making British science policy by stealth

The present British government, noted for (and proud of) its empiricism, appears to have taken an axe (or at least a chopper) to the foundations of British science policy in the past decade without much caring what the consequences will be. Since 1971, the word Rothschild has been (among other things) the code word for a doctrine — that, in the planning of applied research, decisions about what to do and how to spend can sensibly be made only by the ultimate user of the results of the research. But who is the potential user of the results of publicly financed research? Constitutionally, Rothschild recognized, the strict answer must be the taxpayer. In theory, however, taxpayers' interests are represented by government departments. So what more natural than that government departments should be required to work out, between themselves and the Treasury, what proportion of their resources should be spent on research and then to arrange that the work should be undertaken by the contractor offering the best terms? This was the argument accepted by Mr Edward Heath's government in 1971. Different fractions of the budgets of the Agricultural and Medical Research Councils and the Natural Environment Research Council were transferred to the appropriate sponsoring ministries. (The budgets of the Science Research Council and of the Social Science Research Council were, for a supposedly provisional period, left untouched.)

Now (see opposite page) the system is beginning to break down. The Department of Health and the Medical Research Council appear to have decided that Rothschild's omelette had better be unscrambled. The attractions to both parties are clear enough. The department will rid itself of some tedious administration (and may in due course get rid of even more). The council will grow modestly in size (which is probably not an important consideration) and, more to the point, will know more accurately where it stands financially from year to year. Undoubtedly a great many things would, under the proposed transfer of funds, be carried out more efficiently than in the past. It is, for example, anomalous that the Department of Health should have commissioned the study of the side-effects of the use of pertussis vaccine directly from a group of academic physicians, while the council has on its staff or on its books a galaxy of distinguished epidemiologists better able to plan a study of the kind required than almost anybody else. Plainly, part of the weakness of the Rothschild doctrine has been that in some fields of enquiry there is not merely just one customer but just one contractor as well.

For the council, there is only one serious drawback in the proposed arrangements. If the department is planning to hand over at least half the money it now spends on applied medical research, it is only right that the council should accept the responsibility for deciding how these should best be spent. In other words, the council will have to work out for itself some way of identifying the needs of the department and ultimately of the National Health Service. For what it is worth, one of the council's

weaknesses in the heyday of its growth in the 1950s and 1960s was its stolid disinclination to take responsibility for such problems. Now, plainly, its inclinations have changed. It is to be hoped that it will succeed. Certainly a failure to do so will mean further upheaval. On balance, however, there is every reason to hope that the council will be able to rise to the occasion and be the better for having part of its interest in the long-term development of applied research however untidy it may be.

The drawbacks, such as they are, will be found principally at the Department of Health but also in the principles on which public policy on research now rests. Evidently — this is the experience of the past decade — the Department of Health has been at a loss to know how to function efficiently as a customer for research. To use the Rothschild imagery, the department has had money to spend but has not known clearly what it wanted to spend it on. At this stage, it is probably too late to know whether the department has deliberately (or even unconsciously) dragged its feet in setting up the "strong" chief scientist's department that was an essential ingredient of the Rothschild prescription. It is, however, a curious turn-up for the book that the council should now be thinking of taking on responsibility for operational research in health care, apparently because the department does not keenly sense the operational need of the health services. Rothschild would say that that is precisely what is wrong. The trouble with the traditional departments is that they consider research to be a kind of commodity, like string or sealing-wax, that can be bought in from time to time as the fancy strikes the administrators and as the funds allow. Part of Rothschild's point was that government departments should be forced to take a more constructive interest in the potential of research. That battle is on the way to being lost.

The consequences of this new development for the British government's wider conduct of research are harder to foresee. No doubt the administration will be saying that the special arrangement for medical research does not mean that the Rothschild principle has been breached, but the members of the other research councils will be less than human if they do not now sense a quickening of ambition, a tendency towards what the Soviets call revisionism. The Minister of Health, when he comes to describe the new deal in the House of Commons, should therefore be asked to explain why medical research is such a special case. That may be more difficult than he thinks. For although agricultural research appears to have fitted well into the Rothschild framework, it has for some time been clear that the application of the principle to the affairs of the Department of Industry is far from satisfactory. The "requirements boards" intended to simulate customers for research are not a great success. The lack of an obvious contractor has been a serious drawback. Sadly, the time may be approaching for yet another inquiry into the management of research in Britain.

Reorganization of UK medical research

MRC recovers funds from Rothschild?

A plan for reversing the Rothschild disposition of funds for British medical research is to be considered by the Medical Research Council (MRC) at its meeting today (23 October). The proposal, which has already been agreed by the Cabinet and was notified to the Advisory Board for the Research Councils on Monday of last week, may involve an increase of £12 million a year in the annual budget of the MRC, now running at £56 million a year.

Both the Department of Health and MRC at the official level appear to have arrived amicably at the proposal now being decided. One argument in its favour has been the difficulty experienced in the past several years in operating an effective Chief Scientist's Department within the Department of Health. Officials of MRC have also been swayed in the direction of the proposed arrangement by the recognition that, over the past decade, the funds available for contract work from the Department of Health and the Scottish Home and Health Department have been a declining proportion of the total budget for medical research in Britain.

The council will be expected at its meeting today to strike a balance between the benefits of a larger annual budget and the commitment it will now be expected to make to applied medical research. The proposal being considered involves no explicit strings, but representatives of the Department of Health will expect to be listened to more seriously in future if the council elects to take the funds now offered. If the council accepts, the Minister of Health intends to announce its decision

J.L. Gowans, MRC secretary



next Tuesday in the House of Commons.

Formally, the Department of Health cannot off its own bat transfer funds from its own budget to that of MRC, which is dependent on the Department of Education and Science for its annual budget. This is why the feasibility of the proposed transfer of funds has had to be approved by the Cabinet before it could carry conviction with the council. But the redistribution of funds also entails an issue of principle — the abandonment of Lord Rothschild's "customer-contractor principle", at least as far as medical research is concerned.

The Rothschild doctrine, made public in 1971 and promptly accepted by the Conservative government of the day, recommended that 25 per cent of the then MRC budget should be progressively transferred to the Department of Health in the succeeding five years and that the department should use the funds concerned to commission research, not necessarily from the MRC.

One prominent feature of the Rothschild recommendations was the suggestion that each government department should be equipped with a "strong" chief scientist's department capable of making informed judgements of the department's need for research and of deciding how (and by whom) these objectives would best be met.

At the outset, MRC was more vigorous than the other research councils in its opposition to the Rothschild proposals. MRC also stands out among the research councils for the difficulties it has found in

working out a smooth relationship with its sponsoring departments. Committees set up to suggest promising directions for applied medical research have been frustrated either by the lack of funds or the imprecision of their terms of reference. Research commissions by the Department of Health to MRC have generated uncomfortably large files of paper, while the council has been left with the sense that a substantial part of its annual spending is soft money.

At the Department of Health, difficulties have arisen because of the lack of full-time officials with a background in research. The chief medical officer has statutory responsibilities for the health of the population, and may find a conflict of interest between his day-to-day responsibilities and the planning of research. The National Health Service, presumably the prime customer in Rothschild's sense, is, however, administratively separate from the Department of Health proper.

An affirmative decision by the council at its meeting today will require that MRC equip itself to plan and execute programmes in applied medical research ranging from clinical research to the conduct of social surveys related to the effectiveness of medical care in Britain. The prize for success could well be a budget enlarged not merely by the £12 million of Rothschild money but a substantial slice of the further £14 million spent each year by the Department of Health and the Scottish departments on commissioned research with contractors other than MRC.

Genentech makes splash on Wall Street

Washington

"One of the most spectacular market debuts in recent history." That was how the *Wall Street Journal* described the first day of public trading in shares in Genentech, the San Francisco company which has been among the leaders of those aggressively pursuing the commercial exploitation of recombinant DNA technology.

Initially the company has proposed to offer one million shares at between \$20 and \$30 each. But demand was so great that an initial price of \$35 was fixed for members of an under writing syndicate through which the shares were made public last week. An extra 100,000 shares were made available, providing the company with an investment — once brokerage commissions had been deducted — of \$36 million.

During the day of frantic over-the-counter trading, in which more than half of the shares were resold by their original purchasers, the price rose at one point to \$89 a share, eventually dropping back to \$71. As the company has 7.5 million shares, this gives it a value of more than

\$500 million.

On paper, the Wall Street dealings have made multimillionaires of Genentech's two founders, Mr Robert Swanson, its president and chief executive, and Dr Herbert Boyer, vice-president and professor of biochemistry at the University of California, San Francisco.

Both own just under a million shares in the company. The principal shareholder is Lubrizol Incorporated of Cleveland, which previously bought 1.5 million shares in the company at \$10 each. Kleiner and Perkins, an east coast venture capital firm, hold almost a million shares. The remainder are divided between directors and employees of the company.

Some employees have benefited from originally being paid in shares rather than cash. Robert Scheller, for example, a graduate research student at the California Institute of Technology, was given 15,000 for helping with research on human growth hormone four years ago. The stock is now worth more than \$1 million.

The prices reached by the shares during the first day of public trading were con-

siderably higher than most market analysts had anticipated. Two factors contributed. The first was a widespread expectation among investors that genetic engineering is the cornerstone of a future billion dollar industry, and Genentech is the only one of half a dozen small research companies to have gone public.

The second factor was a recent change in the tax law which has helped to restimulate the supply of venture capital. In 1969, Congress introduced legislation raising the effective rate of tax on long-term capital gains from 25 per cent to 48 per cent.

Many investors moved from the stock market to tax shelters, one result being a virtual drying up of new high-technology firms in the mid-1970s. In response, Congress cut the long-term capital gains tax back to 28 per cent in 1978, contributing significantly to the fact that the number of new companies is increasing rapidly again.

Thus, despite recent government warnings about declining investment in technological innovations, there was no shortage of bidders for other Genentech stock. Many brokerage houses were unable to obtain any shares at all in the initial allocation; others who had requested 50,000 or more only received a few hundred.

At the same time, federal officials have taken steps to try to prevent the shares from being oversold, frightened that a backlash could cause investment capital to dry up again. Thus the public offer of the Genentech shares was held up for several days, apparently after a ruling from the Securities and Exchange Commission that more details about the company's operations should be made public.

A revised prospectus issued by the company revealed that in the first half of 1980, Genentech had a revenue of \$3.8 million, of which most came from research contracts, and earnings totalled only \$80,000. Almost two-thirds of the contracts came from three pharmaceutical companies — A.G. Kabi of Sweden, Eli Lilly and Hoffman La Roche — with whom Genentech is working on human growth hormone, insulin and interferon respectively. The prospectus also revealed that the three companies will not be required to pay any royalties to Genentech after their research agreements expire. Although the agreements are described as "long-term", no further details are given.

Although several Wall Street analysts have claimed that the enthusiasm for the Genentech shares showed that the stock market was reviving, others added notes of caution. Half-way through the first day of trading, shares dropped from \$80 to \$74 following warnings from a senior partner of a major investment house that the explosive activity might be a "danger signal" and that "it will attract issues of lesser quality where an attempt will be made to create the same aura of scarcity".

David Dickson

European universities

No room below

European universities seem uniformly to be heading for demographic trouble. According to a 15-nation study commissioned by the European Science Foundation (ESF), European universities have too many academics in the younger age groups and consequently too small a rate of recruitment to university staffs. To make things worse, there is a prospect of falling student numbers as a consequence of low birthrates since 1970.

The report urges "concrete corrective action" to avoid an "irreparable" loss of research talent in the immediate future and, in the longer run, a shortage of trained scientists for the economy. The rate of new recruitment to most university teaching and research staffs is estimated at less than half the 3 per cent per annum required to maintain a flat age distribution.

The decline of the European birthrate since 1970 is potentially a political threat to the universities. The report points out that unless a greater proportion of the age group elects for university education, enrolments will fall, teacher-student ratios will rise and governments will be tempted to cut university budgets.

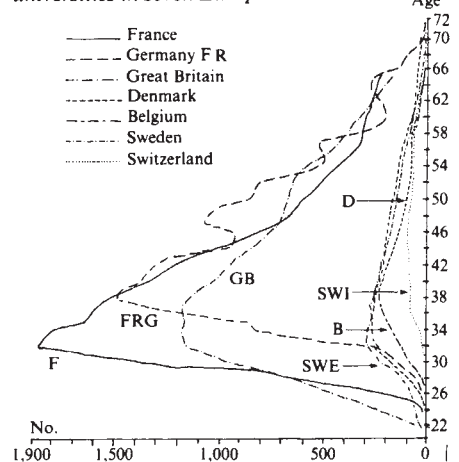
Superficially, France may be the most seriously affected, with the most unbalanced age distribution among present academic staffs (see graphs). In 1977, 46 per cent of French university teaching staffs were aged 30–40, and retirement rates will average only 1.3 per cent a year over the next five years. But France is also among the most active in correcting the problem. In 1975, the Centre National de la Recherche Scientifique set itself a target of creating 3 per cent new posts a year within a staff of some 8,000 researchers. After a shaky start in 1978–79, recruitment seems likely to rise once more to 3 per cent, with consequent benefits for mobility among scientists.

Switzerland is at the other end of the spectrum. Retirements from the universities generate a 2.3 per cent annual demand for replacements in 1981–85, and thereafter more than 3 per cent.

In Germany, replacement demand is estimated at 1.5 per cent a year between now and 1985. The falling birthrate — down by 40 per cent in the period 1967–77

— will be especially troublesome. The "Heisenberg scheme" introduced in 1978 to increase the academic pool by 1 per cent a year, leaves much to be desired. Pay is relatively poor, contracts terminate after 5 years and in any case the most able get the few professorships on offer and leave the scheme.

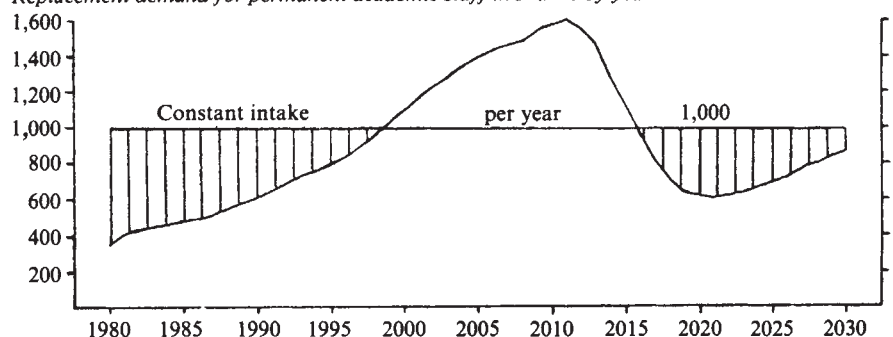
Stock of permanent academic staff universities in seven European countries



In the United Kingdom, the annual replacement rate is expected to be around 1.6 per cent, not reaching 3 per cent until the end of the century. Some 54 per cent of UK university staffs are between 30 and 45 years old. But, like France, the United Kingdom — and particularly the Science Research Council — has been at pains to do something about it. This year, the SRC is expected to make available 15 "Special Release Fellowships", which will pay professional salaries to senior academics — often burdened by administration — to return to full-time research, while the monies thus released to the university will be used to employ a larger number of young researchers.

Of other countries studied, Belgium is in a particularly difficult position: 43 per cent of its university scientific staff are on short-term contracts (compared with 61 per cent in government research institutions) and the majority of these are under 30 years old. Replacement demand for permanent positions is around 2 per cent, higher than most countries, but ESF has discovered no existing schemes in Belgium to tackle scientific unemployment. Denmark, with similar problems to most other countries, has proposals but no action; and Sweden,

Replacement demand for permanent academic staff in France by year.



Norway and Austria provided no information to ESF about the measures they were undertaking.

The net result must be a fair degree of pessimism about the future of academic employment in Europe. The report makes 19 recommendations, few of them novel. The report also makes it plain that scientific mobility is also very low in Europe compared with the United States. Anxiety about jobs seems to have kept people at home. In the United Kingdom, for example, successful applications for the Royal Society European Fellowship exchange scheme fell from 91 in 1976 to 61 in 1979 (although the reverse traffic has kept up at about 90). **Robert Walgate**

Carcinogen regulations

US labs exempt

Washington

Following protests from both university and industrial scientists, the Department of Labor's Occupational Safety and Health Administration (OSHA) seems to be softening its stand on the control of toxic substances in research laboratories. Until recently, OSHA had been insisting that laboratories should be treated no differently from other workplaces in which individuals are occupationally exposed to hazardous substances.

A case in point is OSHA's new cancer policy, which was introduced at the beginning of this year: once a substance has been classed as a carcinogen based on defined scientific criteria, measures must be taken to reduce exposure levels to the lowest that is technologically feasible — and eliminate them if possible.

Labour unions have supported OSHA's argument in the past that laboratories cannot be considered any safer than factory environments, and that the same regulations should therefore be applied to both. Union officials point to reports in the scientific literature which suggest that laboratory workers may be at increased risk with respect to carcinogens, and that cancer rates are high among certain professions, particularly chemists.

University scientists, however, have complained that regulations designed to reduce exposure to chemicals in an industrial environment may be inappropriate and unnecessarily expensive when applied to research laboratories using the same substance.

Additional problems arise from the fact that laboratory work may involve exposure to small quantities of many different chemicals, and that some of these might be difficult to subject to rigid classification.

Although OSHA's new cancer policy received much comment from the chemical industry when it was first proposed, little was heard from the scientific community until the public review process was well under way. (Many laboratories were unaware of the proposals' application to

them.) As a result, when the revised policy was published in January, no specific attempt was made by the agency to exempt research laboratories from its scope. In a preamble to the policy, however, OSHA said that consideration would be given to setting separate standards for laboratories.

Now, under growing pressure from various agencies, including the National Science Foundation (NSF) and the Office of Science and Technology Policy (OSTP), the agency has begun to explore ways of moving in this direction. OSHA officials are working on a proposal, due to be published sometime in the new year, setting out possible procedures for the control of toxic substances in laboratories.

Their proposals are likely to conform closely to the recommendations of the *ad hoc* committee set up by the National Research Council of the National Academy of Sciences (NAS) to look at possible alternatives to the original OSHA cancer proposals as well as other aspects of handling toxic substances. Publication of the committee's report is expected soon.

The committee, initially financed by the American Chemical Society, the Alfred P. Sloan Foundation and the Manufacturing Chemists Association, with later federal support from the NSF, the National Institutes of Health (NIH) and the Environmental Protection Agency, is expected to propose that emphasis be given to developing general laboratory safety regulations rather than the substance-by-substance regulations favoured by OSHA for industrial settings. The committee has in particular been keen to explore approaches which would be more flexible and less costly than those required under OSHA's present regulations.

Also due for publication in the near future are new guidelines that have been prepared by NIH covering the use of potential carcinogens in in-house laboratories. According to Dr Emmet Barkley, director of NIH's new Office of Research Safety, the guidelines (which include provisions for safety plans and medical surveillance) would be triggered whenever either OSHA regulates a chemical or an institute determines that a chemical is a carcinogen.

NIH's guidelines could be used as a model for other research laboratories, if OSHA sticks to a substance-by-substance form of control. However if — as seems more likely — greater emphasis is placed on general laboratory safety guidelines, the NAS report would probably provide the starting point, since this strategy would be a major departure for OSHA.

Another idea on which OSHA is thinking of asking for public comment is the setting up of an advisory committee to comment specifically on the safety measures required in research laboratories. More controversial is likely to be the role played by labour unions, which have been minimally involved in negotiations so far.

David Dickson

1980 Nobel prizes

The Nobel Foundation has in the past two weeks announced in Stockholm the names of the recipients of Nobel Prizes as follows:

Chemistry

Dr Frederick Sanger (MRC Laboratory for Molecular Biology, Cambridge, UK) for the development of a technique for obtaining nucleic acid molecules.

Dr Walter Gilbert (Harvard University, Cambridge, USA) for the development of a different technique for obtaining the sequence of nucleic acid molecules.

Professor Paul Berg (Stanford University, California) for "research in nucleic acids and genetic manipulation".

The Sanger and Gilbert techniques have turned out to be complementary. Sanger uses a single DNA strand to synthesize random lengths of complementary DNA; Gilbert's technique entails the degradation of single strands of DNA in such a way as to generate a mixture of random polynucleotides always including some fixed recognition point.

Professor Berg is known to have been the first to use naturally occurring enzymes to synthesize composite DNA molecules in which two pieces of natural DNA were spliced together.

Medicine and physiology

The prize, which is shared three ways, was awarded for different aspects of the research leading to present understanding of the human histocompatibility gene system (HLA). The recipients are:

Dr George Snell (Jackson Laboratory, Bar Harbor, Maine), responsible for the recognition of the mouse analogue of the HLA system, known as H2, and for the development of appropriate strains of inbred mice.

Professor Jean Dausset, (University of Paris) whose chief contribution was the recognition of the human histocompatibility antigens.

Professor Baruj Benacerraf (an Argentinian working at Harvard University) was chiefly responsible for the identification of the system of genes responsible for the HLA antigens.

Physics

The 1980 physics prize has been awarded for the discovery of what is called "CP violation" to **Professor J.W. Cronin** (Chicago University) and **Professor Val Fitch** (Princeton University).

Their discovery was the experimental observation of the decay of a neutral meson into two charged pions (*Phys. Rev. Lett.* 13, 138; 1964). The outcome was the recognition that in the interactions or spontaneous transformations of elementary particles, parity (left or right-handed geometrical symmetry) need not be conserved.

Almost 8,000 scientists, including 32 Nobel laureates, have signed a "personal moratorium" pledge in support of Andrei Sakharov, Yuri Orlov and Anatolii Shcharanskii, it was announced last week. The moratorium pledge was organized by the "SOS" campaign — original "Scientists for Orlov and Shcharanskii" but extended in January to include Sakharov on his exile to Gor'kii. All three were linked with the work of the Helsinki Watch Committee.

The pledge, which replaces an earlier "personal boycott", binds the signatories not to visit the Soviet Union nor to receive Soviet scientists in their laboratories from 15 May 1980 (the second anniversary of the Orlov trial) until the end of the forthcoming Madrid review conference on the progress of the Helsinki process — unless, in the meantime, Orlov and Shcharanskii are released from labour camp and Sakharov is restored to full liberty, including the right of residence in Moscow. A total of 6,100 scientists and engineers in 44 countries have signed the SOS pledge, while similar but independent pledges in France and Switzerland have so far attracted 1,200 and 600 signatures



Orlov tee-shirts on show

The organizers now plan to deliver an information dossier to the delegations of the 33 countries signatory to the Helsinki Final Act before the conference officially opens on 11 November.

According to Dr John Charap (Queen Mary College, London) who took the chair at a meeting in London last week to report progress, the moratorium and previous boycott pledges have significantly cut Soviet exchanges with the West. In 1979, he said, short-term US-USSR exchanges of personnel had numbered 783 US and 662 Soviet scientists. In the first half of 1980, partly as a result of the pledges, these figures had fallen to a mere 72 US and 117 Soviet scientists.

Since the pledges are essentially personal, it is impossible, of course, to stop Soviet scientists visiting Western establishments, even if there has been a large response to the campaign by the scientists employed there. CERN, for example, still receives a significant number of Soviet visitors, although a large number of the scientists there have organized and signed their own pro-Orlov moratorium. Here, the signatories to the pledge simply refuse

Ariane failure known

A faulty fuel injector in one of four rocket motors led to the failure of the second launch of the European Ariane rocket on 23 May this year.

This is the conclusion reached by the European Space Agency, which said last week that the fault caused the rate of burning fuel in the engine to oscillate 2,000 times per second soon after the launching.

A review group of independent experts has recommended that the Société Européenne de Propulsion (SEP), which built the engines, should make future injectors to finer tolerances, and select good ones by ground firing tests before they are fitted to a launch vehicle.

The injector mixes fuel with oxidant at the top of the engine. It consists of a cylinder equal in diameter to the engine, with 5-cm-thick walls pierced by hundreds of angled holes which direct streams of oxidant onto streams of fuel. Variations, within manufacturing tolerances, of the geometry of the holes seem to be capable of inducing instabilities in the burn — although the fluid dynamics of this is still not understood.

Experiments with engines at SEP in Vernon, near Paris, showed up the fault. Apparently, manufacturing tolerances have slipped since the 200 test firings, although with hindsight low amplitude oscillations have now been discovered in the data.

The third launch — with ground-tested injectors — is now scheduled for mid-March 1981. The fourth and last test flight would be in June 1981, and the first operational flight in October the same year, if all now goes well. The programme would then proceed with the dates planned before the failure of the second launch.

Robert Walgate

to conduct the visitors around their laboratories. When such visitors are expected at CERN, laboratory walls and notice boards display posters bearing simply Orlov's photograph and name, while many staff don T-shirts with the slogan "Free Y. Orlov".

The organizers of the moratoria emphasize that they are not "cold warriors". Nobel laureate H. Christian Anfinsen of the US National Institutes of Health said last week "We have traditionally pushed for togetherness but now we are deciding to hold back because of our concern for human rights".

All moratoria so far have been partial. They do not bind the participants, for example, not to speak to Soviet scientists at international conferences outside the Soviet Union, or not to correspond with Soviet colleagues. Several signatories report tacit sympathy for the campaign among Soviet scientists.

What the organizers hope for is not, however, entirely clear. They stress the

harm that Soviet science will suffer, although the Soviets, in their turn, stress that Soviet science is self-sufficient and that moratoria can only harm the West. Dr Charap said last week that the Soviet government needs Western contacts in order to build up its economy, and that such contacts and exchanges are dealt with under "Basket two" of the Helsinki accords. If the Soviet Union wishes to benefit under "Basket two", he said, it must be prepared also to implement "Basket three", which deals with human rights.

The Western attitude to the Helsinki Final Act has always been that the detente process is indivisible, while the Soviets have preferred to deal separately with the three "Baskets" and especially "Basket one" (arms control). The public speaking of the moratorium organizers is intended to stiffen the Western stand at Madrid on the human rights issue.

Last week's simultaneous meetings in London, Washington, Geneva and Paris were, however, timely for another reason. A few days before, Shcharanskii's mother, Mrs Ida Milgrom, reported that her son had collapsed on 23 September in his Perm labour camp and had been rushed to the prison hospital for emergency treatment of abdominal pains. And in London, John McDonald, QC, announced that Orlov had been put into the punishment area of his labour camp in the Urals, apparently for complaining that he was not receiving his mail and that the guards, drunk on duty, had beaten him up. His right to a visit from his wife this August was withdrawn, and she will not now be able to see him until August 1981. However, said Mr McDonald, Mrs Orlova is afraid that her 57-year-old husband will be unable to survive the present six months punishment regime.

Vera Rich

Soviet science

Relations forsworn

Old wounds will be opened at the salt domes of Gorleben, Lower Saxony, early next year when a new inquiry begins on the disposal of spent nuclear fuel. But this time it will not be a staged confrontation, as happened last May, of nuclear and non-nuclear experts. Now the licensing authority, the Physikalisch-Technische Bundesanstalt of Braunschweig, will present the case from the platform and protesters will have to speak from the floor. And protesters will not be getting financial support from the state.

The previous inquiry led to the rejection of the proposals, for reasons which the prime minister of Lower Saxony, Herr Albrecht, insists were political, not technical. The plan then was for a final disposal facility for all West German nuclear waste, but this has now become a plan for the temporary storage of up to 1,500 tonnes of spent fuel in transport con-

tainers, constantly cooled in a deep pit. A similar proposal is being made at Ahaus, 100 km from Gorleben, but the inquiry there will take place later.

Both proposals are backstops, not permanent solutions to the problem of nuclear waste in Germany. The spent fuel ponds of the existing 12 GW of nuclear power plants are full (allowing space for emergency core dumping), and spent fuel is being transferred to the French reprocessing firm Cogema. But that 500 times per year contract expires in 1984, when French capacity will be fully committed on domestic work.

One alternative being considered is what is called compact storage at existing ponds, using barium shields to absorb neutrons and to kill potential fusion chain reaction. The result could be a five- to sevenfold increase of storage capacity but only in the face of political opposition. As part of nuclear power station site construction (or modification), compact storage must be approved by public inquiry.

Compact storage has nevertheless been agreed for the six nuclear reactors now under construction, which will bring German nuclear power capacity to 21 GW by 1984, so that the issue is only relevant for existing reactors but is there critical. Legally, reactors whose fuel cannot be disposed of safely must be shut down. With the possible ending of the Cogema contract in 1984, West Germany may have to shut down 12 GW of nuclear power.

The sites of Ahaus and Gorleben are thus safety valves, and the planning process has been started in good time in deference to the inevitable opposition. At Gorleben, the chief opponent will be Helmut Hirsch, who organized the opposition at the previous inquiry. Backed by a consortium of environmental groups, he will be arguing that extended storage in transport casks is unsafe.

"The whole scheme is improvised" he said last week. Two years ago, he claimed, the nuclear industry had admitted that there was no recognized system of safe dry storage. In the new proposal, the fuel would become hotter in ponds, perhaps hotter even than in a reactor, and the mechanical stresses involved would lead to corrosion and collapse of the fuel cladding. Hirsch also emphasized that the containers will have to be actively cooled, so that safety will depend on the continuity of the power supply to the plant. The nuclear industry counters that Hirsch's scenarios are extremely improbable.

The long-term deep storage of reprocessed and glassified high active waste seems still a long way off. Indeed, the prospect has become even more uncertain since the last inquiry, for the East German government has now weighed in with complaints about the risks of siting a deep storage facility close to the border, where the prevailing winds would carry emissions to the East rather than the West.

Robert Walgate

Baltic oil pollution Finns fight spill

Finland's Department of Environmental Protection is to set up a new marine biological research and rescue programme to deal with oil spills. This announcement by the Ministry of the Interior follows publication of a report on the damage caused by the oil slick from the *Antonio Gramsci*, which went aground in the Gulf of Riga in February 1979. Although the damage on this occasion was relatively slight, the report found Finnish preparations inadequate to deal with a major slick disaster. The new programme, which will cost an estimated FM 400,000 (£57,000) to implement, will include both oil-fighting and clean-up measures and longer-term research on toxic after-effects.

The oil from the *Antonio Gramsci* first entered Finnish waters at the end of March 1979, striking the edge of the sea ice just south of Utö. It then drifted away towards Sweden, but returned at the end of May and came ashore in the Åland Islands until

4 June. After emergency clean-up measures, a survey was made during July and August on the impact of the oil on fish, seal and bird populations, and on the littoral and benthic ecologies.

Some 1,200 sea birds had perished in the disaster, and a further 500 were soiled. By the time the survey was made, fish life was found to be virtually unaffected. (The islanders had apparently continued to eat the fish without ill-effects.) However, ichthyologists found a significantly higher rate of abnormal tails in the fish examined, although it was not clear that this was the result of oil from the *Antonio Gramsci*.

There was, however, considerable benthic deposition of oil, with the consequent threat of accumulation in food chains. Six months after the incident, there were still traces of oil in mussels.

Finland's unpreparedness for post-spill rescue and clean-up measures is in remarkable contrast to its concern with pollution prevention. The pride of the Finnish passenger fleet, the *Finnjet* ice-breaker ferry, operates as a totally closed environment, with special port facilities for the discharge of sewage and bilges, while her onboard sewage treatment plant has been estimated to cost some £1,000 a day in lost cabin/cargo space alone.

Moreover, under the terms of the Helsinki Convention for the Protection of the Marine Environment of the Baltic Sea, Finland has special responsibility for pollution due to oil spills. Although the convention only came into force on 3 May 1980, there has been joint research and monitoring programmes between the participant countries for several years. A major programme for open-sea monitoring of biomass as an indicator of pollution is already under way. Finland, however, like all other participant countries, is experiencing some delays in getting started; in Finland's case, this is due to lack of carbon-determination facilities in institutes on the coast.

One country which is not taking part in the biomass monitoring programme, although it is a signatory to the Helsinki Convention, is the Soviet Union. The reason for this is mainly that the biomass working group needs to be able to meet at relatively short notice, shorter than the time needed for Soviet experts to obtain visas to attend. Therefore, when, at a working group meeting at the Gdynia Sea Fisheries' Institute last May, monitoring of the Baltic was partitioned between the participant countries, the Soviet offshore waters became the responsibility of Finland and the GDR.

It would seem, furthermore, that the Soviet Union is unable to meet its full commitment regarding damages from the *Antonio Gramsci* affair. The Finns set the total bill at some 15 million Finnmarks, but have agreed to settle for about 25 per cent of this sum, the one million rubles which is the maximum compensation permitted under Soviet law.

Vera Rich

Frosch moves on

Washington

Dr Robert Frosch, administrator of the National Aeronautics and Space Administration (NASA) since 1977, is the latest to join the exodus of science administrators from the federal government.

Following the departure in June of Dr Richard Atkinson, director of the National Science Foundation, and the widely expected appointment of the President's Science Advisor, Dr Frank Press, as president of the National Academy of Sciences (NAS), Dr Frosch has announced that he will become the first president of the American Association of Engineering Societies. on 20 January next year.

Before joining NASA he was associate director for applied oceanography at Woods Hole Oceanographic Institution, and has also been Assistant Secretary of the Navy for research and development. His three and a half years at NASA have seen the agency struggling increasingly against the escalating costs of the space shuttle programme, which is now biting deeply into the space research budget.

Dr Press's appointment is almost settled. His was the only name put forward by the academy's nominating committee, and no serious challenge is expected. Dr Press's appointment would have to be reviewed by a federal ethics committee, established last year to consider the exemption of science administrators from a new law forbidding an ex-government employee from accepting funds from his old agency for two years after leaving government service, but this is not expected to raise difficulties.

David Dickson

CORRESPONDENCE

Science in Spain

SIR — The present status of science in Spain is unworthy of an advanced society that values its standing in the community of nations. It is incumbent upon a modern state to develop a balanced policy of support for basic and applied science; a policy for the advancement of science must be the concern of government as much as economic, educational, foreign and military policy. Unfortunately our administration is derelict of this obligation; it is disregarding the creative aspects of science in favour of short-term pragmatism, typical of a colonial society.

As a matter of intellectual dignity, of national prestige and of responsibility to future generations, this situation must not persist. As Spanish scientists we demand that the administration respect our right to pursue our scientific interests, and we assume the responsibility for generating a scientific establishment that is both of value to our society and a credit to our country.

The efforts committed to this endeavour will be justifiable only if the resulting science and technology are competitive at the international level. In order to be competitive, technology must be original, and because of its modern complexity it depends on complete and continuous access to basic research. It is impossible to generate a competitive technology without a parallel reinforcement of research in basic science.

The extent of the task that confronts us can be evaluated by examination of the commitment to science in other countries in the European Community. The initial investment required to approach their levels is modest, both in absolute terms and in comparison with the cost of other national programmes. However, this effort must begin immediately, because the deterioration of our scientific institutions, the demoralization of our scientists and the loss to other countries of the most able, especially among the young, increases daily.

In order to invigorate our science, the university departments must become centres of scientific excellence, with a balanced dedication to preparing new professionals and to research. Moreover, because of the complexity of modern scientific investigation it is necessary not only to reorganize and strengthen existing research centres, such as the Research Council, the Nuclear Energy Commission and the Agricultural Research Institute, but even to create new ones.

Our country cannot be expected to reach a satisfactory level of cultural and economic development or a minimum of political independence, if we do not soon recognize that progress depends on knowledge. State administrators must become aware of the necessity of stimulating sound scientific research and of the historical responsibility they have in this endeavour.

The above *Manifesto* has been signed by, among others, 134 professors, 25 associate professors of the University of Madrid, 23 research professors and 14 investigators of the Spanish Research Council, heads of departments or institutes in the areas of

humanities, natural sciences, medicine and technology in nineteen major cities of the country (Barcelona, Bilbao, Córdoba, Granada, La Laguna, León, Llerida, Madrid, Málaga, Murcia, Oviedo, Pamplona, Salamanca, Santander, Santiago, Sevilla, Valencia, Valladolid and Zaragoza) besides 21 investigators, heads of research groups in the Spanish Atomic Energy Commission and several medical institutions.

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Nuclear electricity

SIR — The article 'Nuclear generating costs compared' (*Nature* 21 August, p.753) states that the analysis of relative costs given in the 1979/80 Report of the Central Electricity Generating Board (CEGB) "is likely to be much quoted in the months ahead, when plans are being laid for the further development of the British nuclear power programme". This is certainly true and it is therefore a pity that the report's figures were reproduced without question. In fact, for the purposes of comparison, the figures are almost totally misleading.

The apparent lower cost of nuclear (Magnarox) electricity (1.30 pence per kWh against 1.56 pence per kWh for coal) is entirely an artefact of inflation. If currency had been stable over the lifetime of the Magnox stations (or if inflation had been corrected for in the accounts) the order would have been reversed. In 1980 prices the approximate corrected costs are 2.5 pence per kWh for electricity from nuclear stations and 1.9 pence per kWh from coal-fired stations¹.

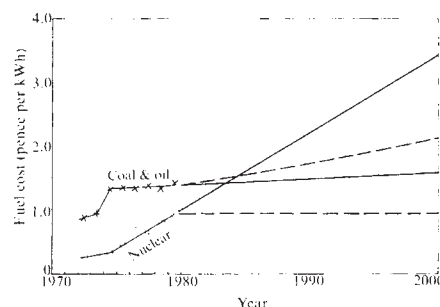


Fig. 1 Fuel costs from the 1979/80 CEGB Statistical Yearbook, Tables 9 and 10, from 1972/3 to 1979/80 in pence per kWh corrected to 1979/80 prices using the Retail Prices Index. Crosses, coal and oil combined; circles, nuclear. 1980–2000 extrapolations: solid line, linear; dashed line, CEGB extrapolation for net effective cost calculations.

The full calculation can only be undertaken when the CEGB decides that its present policy of withholding the data from which Table 1 in the *Nature* article was calculated is counterproductive, but all the indications are that the real cost of nuclear electricity in 1979/80 was well over 50 per cent greater than that from coal-fired stations.

When we come to future costs (Table 3 in the *Nature* article) the figures are calculated in 1980 £'s. But here the figures are equally

misleading for different reasons. Richard Marshall (*Nature* 18 September, p.184) has dealt with the CEGB's optimistic figures in the light of actual operating experience with regard to load factors. An equally optimistic view is taken of nuclear fuel costs relative to those of coal. Figure 1 shows the real costs of nuclear fuel for the last eight years. This rose quite rapidly for the first three years but then shot up at a rate of 0.12 pence per kWh per year to 0.94 pence per kWh in 1979/80. The increase shows no sign of slowing down and if it continues nuclear fuel costs will overtake those of coal in the mid 1980s.

It is significant in respect of future fuel costs that the 1979/80 CEGB Report records with satisfaction that an understanding has been reached with the National Coal Board to take 75 million tons of coal a year provided that the real price of coal does not increase for five years, but "the terms of a contract with British Nuclear Fuels Ltd for reprocessing oxide fuel from the advanced gas-cooled reactor (AGR) stations have yet to be negotiated".

It is extraordinary that the CEGB has nevertheless nowhere, either in its reports or in the evidence to the Select Committee, discussed the alarming increase in real nuclear fuel costs (over 10 per cent per year for the last five years) but has concentrated on postulated increases in the real price of coal up to the end of the century. This hypothetical increase is actually incorporated into Table 3. The figure of 2.38 pence per kWh for 'inclusive fuel costs' of a coal-fired station is composed of two parts. 1.36 pence per kWh is the approximate current fuel cost in March 1980 prices and the remainder, 1.02 pence per kWh, is entirely dependent on the assumption about increases in coal costs up to the year 2000. If real future costs for coal are assumed to remain stable this part would be zero.

No such addition to current costs occurs with nuclear fuel. The figure of 0.61 pence per kWh in March 1980 prices hardly covers the increase due to inflation from the 0.55 pence per kWh given for Hinkley Point B in 1979/80 average prices, let alone any allowance for the rapid rise in real costs. There is no provision for "present value of future real increases in the price of fuel" in the nuclear case and this means that it is assumed there will be no real increases in the price of nuclear fuel over the 25 year lifetime of the station.

It is on the basis of such wild assumptions that the net effectiveness cost calculations over the whole lifetimes of the stations have produced the apparently favourable results for nuclear power.

It is difficult to see why the CEGB did not present the obvious calculation — the estimated cost in pence per kWh for each station in its year of commissioning in 1980 costs and prices — and then make the various points relevant to possible alterations in these costs in the future. But then these considerations would have been explicit and generally understandable, whereas wrapped in the computer program for finding net effective cost they are almost immune from criticism.

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NEWS AND VIEWS

The economics and management of commercial fisheries

from Robert M. May

THE blockade of Channel ports by French fishing boats this summer will have forcibly reminded many unfortunate travellers that managing the fisheries of Northern Europe is no simple task.

As will be argued below, the major problems are economic, social and political rather than scientific. The scientific problems are, however, formidable. To begin, data for fish populations¹ and the intrinsic dynamical behaviour of the populations²⁻⁴ are both subject to many kinds of stochastic effects. Thus, even when the millennium dawns and fisheries managers completely understand the biology underlying recruitment processes, predictions about stock densities and recommended catch levels are likely to remain probabilistic rather than deterministic statements^{5,6}. This probabilistic character infuses much of the biological sciences⁷, contrasting with the crisp determinism of most of classical physics and engineering; a contrast leaving many people with the uncomfortable feeling that such areas of biology are 'unscientific'.

The millennium lies a long way off for fisheries scientists, and many fundamental biological problems remain unsolved. One problem concerns the relation between stock and recruitment¹⁻⁸. For whales, other marine mammals and some fish there is a rough — a very rough — understanding of the relation between the size and age composition of the population and the recruitment rate. But for most fish (including essentially all the commercially important species in the North Sea) recruitment appears to be approximately independent of stock density down to some threshold level, below which recruitment appears to collapse suddenly; at present, there is no *a priori* way of determining this threshold, although it may be (and this is debatable) that it has been discovered *post hoc* in 7 major clupeoid fisheries that have collapsed⁹ (North Sea herring, Norwegian spring spawning herring, Hokkaido-Sakhalin herring, Pacific sardine, Far Eastern sardine, Southern African sardine, Peruvian anchovy). Another set of problems arises from the simultaneous harvesting of several different species that

interact within a complicated ecosystem^{1-10,11}; such problems are becoming more important with overfishing of many conventional fish species and the search for new kinds of food from the sea.

These scientific problems are the subject of several recent reviews^{6,8,11,12}; the book edited by Gulland¹ is particularly good. The difficulties are often compounded by inadequate reporting of catch data. For instance, the International Council for the Exploration of the Sea (ICES) working group on hake stocks this year decided to double Spain's official figure for their hake catch (taken under license in EEC waters), on the basis of the number of unlicensed Spanish vessels sighted in the Bay of Biscay by the French authorities. Moreover, the agreed rules are not always obeyed: again to use hake as an example, French and Spanish fisheries in the Bay of Biscay in 1979 exceeded the total allocated catch (TAC) by some 10,000 tonnes, using small-meshed nets such that 80 to 90 per cent of the catch was below the internationally agreed minimum size of 25 cm. Although the reasons given in defense of such infringement are often that the TAC set by ICES or other international bodies is not scientifically sound (even *Le Monde*¹³ writes of "foreign competition (using) so-called scientific arguments, such as, for example, slashing quotas of catches in authorized fishing areas . . ."), the real reason lies in the economics and politics of the situation. To this I now turn.

In the past, most marine fish stocks have been exploited as 'common-property' resources; fishermen (unlike farmers, miners, or lumbermen) do not usually own the primary resource base from which their income is derived. In such common-property, open-access situations, there ensues what Hardin¹⁴ has called a 'tragedy of the commons', as fishermen are forced to compete for their share of the catch taken from a finite resource stock. Even after the establishment of international management commissions which seek to prevent excess depletion of stocks (or rehabilitation of overexploited stocks) by restrictions on fishing gear, closed seasons and areas, total catch quotas and so on, there remains a basic tension between¹² "the interests of the individual ('will an additional vessel make a profit') and of society as a whole ('will an additional vessel result in an increase in the value of total

catch that is greater than the cost of the vessel'). In this way, competition within common-property fisheries has repeatedly led to overexpansion of fishing capacity and "dissipation of economic rent", even in those cases where management has succeeded in preventing depletion of the fish stocks.

With the introduction of 200-mile Exclusive Economic Zones (EEZ) by coastal states, most of the world's fish stocks are now susceptible to rational management; by the end of 1979, 99 per cent of all commercial fish catches were contained within the new EEZs. The UN Food and Agriculture Organization (FAO) has noted¹² that this global movement to patterns of national "sole ownership" marks "a time of fundamental change and tremendous opportunity . . . to move in an orderly way to develop processing capacity and markets based on a stable supply, and in general to look forward to great and continuing benefits from the resource". Partly motivated by these opportunities, there has been an upsurge in interest in methods for the economic regulation of commercial fisheries^{12,15-19}.

In particular, Clark¹⁷ has analyzed models for fisheries, incorporating the microeconomic decisions made in the operation of individual vessels, in order to understand the consequences of various methods of regulation. These include total catch quotas, vessel licenses, taxes on catch (or effort) and transferable catch (or effort) quotas allocated to individual vessels.

Setting aside the technical problems associated with a licensing programme (who gets licenses, at what cost and under what limitations of transferability, etc.), it is important to realize that licensing by itself does not resolve the common-property problem of fisheries. The usual experience of licensing programmes has been that²⁰ "licensed vessels have been progressively replaced by larger, more powerful vessels with increased capacity for catching and storing fish on board." Such expansion in capacity typically necessitates additional regulation: the size and horsepower of vessels may be controlled, restrictions may be placed on gear or detection equipment, fishing grounds may be closed at specified times, and so on.

Similar problems beset regulation by

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total catch quotas. Both licenses and total quotas can prevent depletion of stocks, but they almost inevitably lead to the kind of overexpansion of fishing capacity that causes many of the problems in the North Sea. As Clark²⁰ summarizes it, "In practice, this process usually manifests itself in terms of a progressive shortening of the fishing season, with the entry of more and larger vessels, which become capable of capturing the annual quota in fewer and fewer days of fishing. When the regulation is by means of gear restriction, closed seasons, and the like, the resulting fleet expansion will tend to overcome the regulation, which will then have to be revised, initiating further expansion."

In short, any regulatory process that leads to fishermen competing for the catch may force individuals to act in ways contrary to the overall economic efficiency of the fishery.

A system that allocates quotas to individual vessels has the fundamental advantage of counteracting these competitive features. Clark notes that such allocated quotas are frequently used to manage other common-property resources, such as water supplies and grazing land²⁰: "The owner of a quota knows that (barring unforeseen circumstances) he will be able to take his quota regardless of the activities of other users. On the other hand, he is prohibited from exceeding his quota. With this knowledge, it is then to his benefit to exploit his quota in the most economically efficient manner. The main incentives for wasteful expansion of fishing capacity and other inefficient activities are thus eliminated."

The quota certificates or licenses should, furthermore, be transferable. They would thus acquire a monetary value, keyed to fish prices and the costs of fishing, which would encourage efficiency and also would²⁰ "resolve the problem of permitting new entrants — although entry would of course be open only to those who

could afford to buy up an existing quota license".

Clark¹⁷ has also given a proof that taxes or royalties on catch or effort are formally equivalent to allocated quotas. In practice, however, this formal result should not be taken too literally; quotas by their nature provide a direct control over catches, and thence over stock density, whereas taxes act indirectly. Control of the total catch rate via taxes or royalties requires knowledge of the price of fish, the cost of fishing effort, and in general depends on the behavioural responses of fishermen, all of which are subject to serious imprecision and uncertainty.

For international fisheries, the allocated quota system may be extended by setting national quotas, which serve to divide fishing rights among the nations involved. Then each nation can apportion its quota, allocating fishing rights to individual fishermen or firms.

There remain, of course, many technical difficulties in the implementation of any scheme of allocated quotas. These include: the set of scientific problems noted above; problems in dealing with the intrinsic variability of many fish stocks; the

management of multiple-purpose fishing fleets and multi-species catches; and the inevitable problems of enforcement. Further complexities enter when one gives explicit attention to the "concentration profile" characterizing the way a fish population is distributed in space or time¹⁸. Thus the economics and population dynamics of harvesting a stock that is uniformly distributed in the sea will differ from those for a stock that aggregates in a relatively few large schools; exploitation will always tend to follow the concentration profile, focusing first on the highest concentrations. Depending on the shape of the concentration profile, this can complicate the economics and management of the fishery.

In general, however, there appears to be a feeling¹² that the establishment of EEZs has provided a "finite opportunity — a window in time — in which the world community and the coastal states can work together to place these new national fisheries on a solid groundwork of management", and that the best available regulatory instrument is a system of transferable catch (or effort) quotas allocated to individual fishermen or vessels. □

Roles of RecA revealed

from Steve Sedgwick

AT first glance it seems improbable that a single protein can perform genetic recombination, regulate its own synthesis, and switch-on expression of several other operons. However it is now evident that the recA protein of *Escherichia coli* has all of these capabilities. Not surprisingly the activity and regulation of this protein are currently under intense scrutiny.

In a series of recent papers, groups led by C. M. Radding¹ and I. R. Lehman² have shown that purified recA protein will stimulate the formation of DNA structures resembling putative recombination intermediates. One such structure is the D-loop where a single DNA strand pairs with a complementary region in a locally denatured section of double stranded DNA. D-loops may represent an early stage in recombination where a single DNA strand invades a homologous duplex to initiate strand exchange. A series of partial reactions, all requiring recA, occur in D-loop formation. At low concentrations recA protein binds to single stranded DNA. At higher concentrations it binds to double stranded DNA using ATP as a cofactor. A three stranded complex then forms in which double stranded DNA is unwound. Finally ATP is hydrolysed and recA protein

dissociates. If hydrolysis takes place at a region of homology between single and double stranded DNA a D-loop can form. Heterologous and homologous single strands stimulate unwinding, perhaps explaining how recA protein seeks for homology between DNA molecules. Unwinding occurs without topoisomerase (nicking-closing) activity and so requires a free DNA end or superhelical turns so that DNA strands can rotate around each other.

RecA protein will also join double stranded DNA circles to make figure-of-eight structures, provided that one of the circles has a single strand gap^{3,4}. A joining reaction analogous to D-loop formation has been proposed where initial pairing occurs between an unwound section of duplex DNA and single stranded DNA in the gap of the second molecule³. Nevertheless, some uncertainty remains whether D-loop formation really reflects an event in recombination between duplex DNA. First, DNA crosslinking does not reduce the yield of figure of eight molecules even though it impairs strand separation and presumably, D-loop formation⁴. Second, binding of recA protein to gapped DNA is not restricted to single strand regions and extends into much of the double stranded portion of the molecule⁵. Howard-Flanders and colleagues suggest that duplex DNAs bound with recA protein can form four stranded synapsis structures⁴.

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They refer to theoretical work showing that a helical arrangement of four DNA strands with four-square base pairing can be formed without strand breakage⁶.

Indications that *recA* protein has a regulatory role, switching-on expression of its own and other genes, first came from observations on the physiological response of *E. coli* to DNA damaging treatments. It was found that the *recA* gene, and a second gene, *lexA*, were needed to induce synthesis of large amounts of *recA* protein and a variety of new physiological responses. The induced responses have been called SOS functions because some aid cellular recovery. Better known SOS functions are the induction of prophages to enter a cycle of lytic growth, induction of new DNA repair systems, induction of mutations, and induction of filamentous growth. Biochemically, λ induction is best understood and occurs by *recA* protein proteolytically cleaving the λ repressor protein⁷. The λ repressor is then unable to bind to λ operator sites and prevent transcription. It has been hypothesised that the *recA* gene, and genes for the other inducible processes, also have repressors sensitive to *recA* proteolysis.

Direct evidence for an array of operons regulated by *recA* and *lexA* has now been presented by Kenyon and Walker⁸. By using a specialized Mu-lac transducing phage developed by Casadaban and Cohen⁹ they made random insertions of the *lac* structural gene into the *E. coli* chromosome. Bacteria were selected which became lactose positive after DNA damaging treatments. These bacteria presumably have a *lac* gene fused into a transcriptional unit whose expression is induced by DNA damaging treatments. So far five different loci have been identified where *recA* and *lexA* functions are needed for induction of gene expression. Surprisingly one of these loci appears to be the *uvrA* gene, a component of the excision repair process, long considered to be distinctly separate from *recA* and *lexA*

pathways of repair.

Extensive genetic evidence indicates that expression of the *recA* gene is switched off by the product of the *lexA* gene, possibly acting as repressor. This is the regulatory state of a normal cell and provides a low basal level of *recA* protein sufficient to perform genetic recombination and recombinational DNA repair. Little *et al.*¹⁰ have shown that *recA* protein switches on expression of its own gene by inactivating the *lexA* protein. *In vitro* they found that *lexA* protein was proteolytically cleaved by purified *recA* protein in a reaction essentially identical to *recA* proteolysis of λ repressor. *Lex A* 3 mutants are unable to induce *recA* protein synthesis and have a *lexA* protein insensitive to proteolysis. It seems that *recA* protease activity is triggered by interaction with a signal molecule, possibly single stranded DNA or a short oligonucleotide, which accumulates in DNA damaged cells.

An additional twist to this story, described by Brent and Ptashne¹¹, is that *lexA* protein switches off transcription from the *lexA* promoter. Thus the *lexA* gene has negative autoregulation. Increased *lexA* protein levels also switch off expression of certain other genes such as those causing filamentous growth.

To summarise, evidence for the following control network is emerging. *LexA* protein represses expression of its own gene, the *recA* gene, and genes for some SOS functions. Other SOS functions are switched-off by their specific repressor molecules. DNA damage activates protease activity in the basal *recA* protein. *RecA* protease cleaves the *lexA* protein switching on expression of the *recA* gene

and SOS genes under the direct control of *lexA*. Other SOS functions are de-repressed by *recA* proteolysis of their specific repressors.

The structural basis of *recA* protease and recombinase activities has now begun to be examined. As a first step the amino acid sequence of the protein was determined from the nucleotide sequence of the *recA* gene, combined with partial sequencing of tryptic fragments¹². The gene contains 1,059 coding bases specifying a 352 amino acid protein with a calculated molecular weight of 37,842. The nucleotide sequence contains several regions resembling 'vestigial' insertion sequences with characteristic direct repetition of 8-11 bases. An intriguing speculation is that the *recA* gene evolved through a process involving transposition. The amino acid sequence indicates the presence of central β sheet flanked by partially α helical segments. The amino terminus contains sequences of amino acids reminiscent of active sites of known serine proteases. It is worth noting that four *recA* mutations primarily affecting the induction of SOS functions were earlier mapped in this region¹³. The carboxy terminus of *recA* protein is rich in aromatic and basic amino acids believed to be important in DNA binding, and so might be concerned with recombination functions. A more detailed understanding of active site domains should come with the sequence analysis of different alleles of the *recA* gene. Finally, the announcement that *recA* protein has been crystallized and subjected to X-ray diffraction analysis promises an intimate dissection of this protein's structure in the near future¹⁴. □

Charon's diameter

from David W. Hughes

OCCULTATION observations have been the mainstay technique for the measurement of planetary diameters for a considerable time now, and the discovery of such things as the Uranian satellite belt in 1977 and a binary asteroid in 1978 are obvious bonuses. The principle is simple. A planet passes in front of a distant star and blocks out the starlight for a specific time. Measurement of that time interval gives the angular size of the chord of the planetary disc in that direction. If this is done from a range of observatory positions on Earth the size and shape of the planetary disc perpendicular to the line of sight can be obtained. The method is technically quite difficult because it relies on extremely accurate knowledge of stellar and planetary positions and the use of highspeed photometers at the focal planes of large telescopes.

Whether or not an occultation of

Charon, Pluto's satellite, would occur was difficult to predict. Gordon Taylor of the Royal Greenwich Observatory reported that an occultation by Pluto or Charon of a 13th magnitude star in Virgo would possibly take place at about 0h on April 7, 1980. Subsequently it was predicted that Pluto would be about 1 arc sec away from the star and no occultation would be seen. Then it was noticed that Charon would be on the 'star side' of its orbit around Pluto and would be within about 0.2 arc sec of the track. As an error in the known position of Charon of ± 0.2 arc sec is quite possible Alistair R. Walker of the South African Astronomical Observatory decided to attempt the observation. His success is reported in a recent edition of *Monthly Notices of the Royal Astronomical Society*, **192**, 47P, 1980.

The occultation was monitored using a 100 cm telescope coupled to a pulse counting photometer which could operate at 1 ms per reading without difficulty. Twin

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channels were used, one looking at the star which was to be occulted, the other at a nearby reference star. The data was taken using an integration time of 2 sec. Sky conditions were excellent and the occultation took place at 23h 39m 28s on April 6, 1980 and lasted for 50 seconds. This duration corresponded to 0.055 arc sec or 1,200 km at the distance of Charon. Unfortunately, no other observations were made in the southern hemisphere (no occultation was observed from England) so the chord length of 1,200 km must simply be taken as a minimum value for the diameter.

Another clue to the size of Charon lies in the fact that it is 1.7 ± 0.1 magnitudes fainter than Pluto. Unfortunately, the

magnitude, which is a direct measure of the brightness, depends not only on the illuminated surface area but also on the reflectivity. There is no specific reason why the reflectivity of Pluto and Charon should be the same. Other satellites are not noted for having similar reflectivities to their planets.

The dominant constituent of the surface of Pluto is methane ice. By assuming that Charon has a similar covering and that its density is greater than 0.35 g cm^{-3} Walker concludes that its diameter lies in the range 1,350 to 1,800 km.

Pluto and Charon thus seem to form a 'double planet' with probable diameters of 3,600 and 1,650 km respectively. The mass ratio is about 10:1, an extremely low value

for the ratio between the mass of a planet and its primary satellite. Whether this is significant or not is a matter for future conjecture but it does bring to mind a point stressed by R.A. Lyttleton (*Mon. Not. R. astr. Soc.*, **121**, 551; 1960) when discussing the origin of the solar system. The fissional break up of a rotating protoplanet results, according to Lyttleton, in two main fragments with equal densities, the mass ratio of these portions being at least 8:1, but not greatly more than this. Unfortunately, Lyttleton points out that the two fragments separate at more than the escape velocity and cannot remain gravitationally bound to each other. So the origin of the Pluto-Charon double planet is still a mystery. □

Opioid peptides: search for the precursors

from Michael J. Brownstein

IN 1975 Hughes and his colleagues unraveled the structures of two opioid peptides — methionine-enkephalin and leucine-enkephalin — that they had isolated from brain extracts¹. They pointed out that the amino acid sequence of methionine-enkephalin was contained within another larger peptide, β -lipotropin², and they suggested that the latter might be a methionine-enkephalin precursor. It is known that β lipotropin does give rise to at least one family of opioid peptides, the endorphins, but, although methionine enkephalin can be

enzymatically liberated from the largest of the endorphins (β -endorphin), recent evidence suggests that both methionine- and leucine-enkephalin are produced by a quite different route.

Methionine-enkephalin and β -endorphin are found in separate populations of cells both in the central nervous system and the periphery. For example, β -endorphin, but not methionine-enkephalin, is found in high concentrations in the intermediate and anterior lobes of the pituitary where it is synthesized as part of a 31,000 molecular weight protein ('pro-opiocortin') that also

gives rise to adrenocorticotrophic hormone and α -melanocyte-stimulating hormone⁵. Again, very high levels of methionine-enkephalin and leucine-enkephalin, but not β -endorphin, are present in chromaffin cells of the adrenal medulla⁶. Finally, it is quite clear that leucine-enkephalin must be synthesized separately from β -endorphin because its amino acid sequence is not present in pro-opiocortin⁷.

In the years since the enkephalins were first described a number of peptides with opioid activity have been discovered. Most of these contain either the methionine-enkephalin sequence (H-Tyr-Gly-Gly-Phe-Met-OH) or the leucine-enkephalin sequence (H-Tyr-Gly-Gly-Phe-Leu-OH).

As shown in the table some of the unique enkephalin-related molecules are generated by incubating adrenal proteins in the presence of trypsin^{10,11}. These include H-Tyr-Gly-Gly-Phe-Met-Lys-OH and H-Tyr-Gly-Gly-Phe-Met-Arg-Arg-OH. In addition, methionine-enkephalin, leucine-enkephalin, H-Tyr-Gly-Gly-Phe-Met-Arg-OH¹², and H-Tyr-Gly-Gly-Phe-Met-Arg-Phe-OH⁹, are found in tryptic digests of adrenal medullary proteins. The larger molecules that give rise to the above peptide species are being isolated and analyzed. In the bovine adrenal two possible precursors with molecular weights of 3,000 to 5,000 have been already described¹³ and are shown in Table 2.

Note that trypsin, which cleaves on the carboxy terminal side of basic amino acid residues, would liberate a single leucine-enkephalin molecule from the upper peptide and a single methionine-enkephalin molecule from the lower one. Trypsin would also release H-Tyr-Gly-Gly-Phe-Met-Arg-OH and H-Tyr-Gly-Gly-Phe-Met-Lys-OH from the upper and lower peptides respectively and methionine-enkephalin could be made from either of these molecules by removing

Table 1. Opioid Peptides

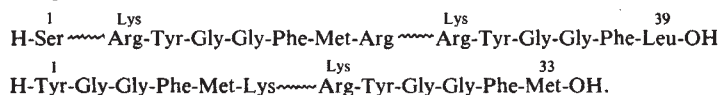
Methionine-Enkephalin Related Peptides			
Trivial Name	Source	Structure	Ref.
Methionine-enkephalin	pig brain	H-Tyr-Gly-Gly-Phe-Met-OH	1
Pro-methionine-enkephalin	Pig hypothalamus, human adrenal medullary tumor	H-Tyr-Gly-Gly-Phe-Met-Arg-OH	12
—	bovine adrenal medulla	H-Tyr-Gly-Gly-Phe-Met-Arg-Phe-OH	9
Molluscan cardioexcitatory neuropeptide (FMRFamide)	bovine striatum clam ganglia	H-Phe-Met-Arg-Phe-NH ₂	8
—	trypsinization of bovine adrenal medullary proteins	H-Tyr-Gly-Gly-Phe-Met-Lys-OH	10,11
—	trypsinization of bovine adrenal medullary proteins	H-Tyr-Gly-Gly-Phe-Met-Arg-Arg-OH	10,11
β -endorphin	camel pituitary	H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln-OH	3
Leucine-Enkephalin Related Peptides			
Leucine-enkephalin	pig brain	H-Tyr-Gly-Gly-Phe-Leu-OH	1
α -neo-endorphin	pig hypothalamus	H-Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Arg-Pro-(Gly ₁ Tyr ₂ Lys ₂ Arg ₁)	15
Dynorphin-(1-13)	pig pituitary	H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH	16

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the arginine or lysine by a carboxypeptidase B-like 'trimming enzyme'.

In human adrenal tumours there are two 2,000 molecular weight peptides that yield methionine-enkephalin when they are treated with trypsin and carboxypeptidase B (see ref. 11). The relationship of these two peptides to one another and to the 3,000-5,000 molecular weight bovine adrenal peptides described above is uncertain. It is clear, however, that 2,000 to 5,000 molecular weight human and bovine peptides are too small to be translation products themselves. They are more likely to be parts of a larger molecule; and, in fact, 8,000 to 50,000 molecular weight molecules have been detected in the brain and adrenal that either bind directly to

Table 2: Two precursor molecules from the bovine adrenal gland which could give rise to enkephalins.



opiate receptors or enkephalin antibodies or do so after treatment with trypsin^{10,13,14}.

Recently, Lewis and his coworkers have isolated a 50,000 molecular weight protein from adrenal medulla that they think is an enkephalin precursor able to produce both methionine-enkephalin and leucine-enkephalin. The molecule contains methionine- and leucine-enkephalin sequences in a ratio of about 7 to 1, and could also contain α -neoendorphin¹⁵ or dynorphin¹⁶ (see Table 2), but (assuming that there is only one mole of leucine-enkephalin per mole of precursor) not both of them. It is also possible, of course, that α -neoendorphin, dynorphin, and methionine/leucine-enkephalin are all made in separate groups of cells and that there are precursors distinct from the postulated methionine/leucine-enkephalin precursor. This could account for the report that there are separate leucine-enkephalin and methionine-enkephalin containing cell populations in the brain¹⁷.

To establish a precursor-product

relationship between the 'big' enkephalins that have been discovered and the small forms, pulse labeling studies will have to be undertaken. Workers in a number of laboratories have shown that radiolabeled amino acids can be incorporated into methionine- and leucine-enkephalin *in vivo* by the rat brain¹⁸, and *in vitro* by striatal slices¹⁹, dissociated spinal cord cells²⁰, myenteric plexus explants²¹, and adrenal medullary chromaffin cells^{22,23}. In addition, Rossier and his colleagues²³ have shown that a 22,000 molecular weight methionine-enkephalin containing protein is synthesized in advance of methionine-enkephalin and may give rise to it. Much more work needs to be done to characterize the enkephalin precursor(s) and to show how they are metabolized into active peptides. The regulation of this process also needs to be studied in detail²².

When the biochemistry, anatomy, and pharmacology of the various opioid peptides are better understood, the reason for their diversity may be more obvious. □

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Big eyes

from M.J. Disney

HISTORY shows that in astronomy, scientific advance rides on the back of technical progress. The last major step forward in optical telescope design was conceived in 1928 and completed in 1950 with the commissioning of the Hale 5-metre telescope on Palomar Mountain. Since then the emphasis has been on light detectors, which have improved 100 fold in sensitivity and which now respond to about 50 percent of the light collected. The scope for further significant improvement is therefore limited, for which reason interest has again turned to attempts to increase collecting areas. To that end a large workshop was held earlier this year at Kitt Peak National Observatory in Arizona.

Astronomers rely on optical spectroscopy to determine the redshift, distance, composition, luminosity, rotation-speed and temperature of sources. The spectral resolutions needed to supply these data require a light gathering capacity hundreds of times greater than that sufficient to merely detect the objects. To obtain detailed information from the clouds of

faint galaxies that we can already see an ambitious increase in telescope size will be required. Many conference participants felt that a collecting area equivalent to a single 25 metre diameter mirror was a reasonable goal at which to aim.

Ideally, such a massive optical telescope should be placed in space where, free of atmospheric seeing problems, it would reach its diffraction limited performance. The 2.5-metre Space Telescope to be launched in 1983 will provide increased spatial resolution but its light gathering power may still not be sufficient for spectroscopic analysis of extended objects like galaxies. As the Space Telescope will cost 500 million dollars the launch of an even larger telescope seems out of the question.

The costs for conventional ground based instruments seems to increase approximately as the 2.6 power of diameter, so that more than 2 billion dollars would be needed for a 25 metre conventional telescope. However, technological developments, particularly

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*The conference proceedings *Optical and Infra-red Telescopes for the 1990's*, will be published in 2 vols. by Kitt Peak National Observatory, ed. Hewitt.

thin mirror construction, polishing of fast (that is highly curved) primaries, computer aided design and control, and the alt-azimuth concept with its favourable affects on dome size, will enormously decrease the costs of future telescopes per metre of aperture. A 2-metre for less than \$2.5m complete is now a certainty and proposals for a 14-metre at the price of a conventional 4-metre are being discussed in detail.

With falling costs, single institutions may be able to contemplate a big telescope for themselves. The University of Texas is considering a thin mirror (20 cm) 7-metre monolith for less than 25 million dollars; the University of California, a segmented 10-metre mirror for somewhat more. Domes can prove expensive items in the budget: at 2 million dollars the rotating asymmetric dome of the 3.6-metre equivalent Multi-Mirror Telescope, now in use on Mt Hopkins, is probably a realistic pointer towards the future.

The high cost of the conventional telescope springs largely from the mass needed to maintain optical rigidity. Very large telescopes must depart from this philosophy and rely instead on the active interrelation of light thin structures. The reflecting area will either be segmented or divided into a number of separate mirrors aimed at a common focus: lasers may be used for active alignment of the components (a scheme already being tested on the Multi-Mirror Telescope) although simpler, computer-based, electromagnetic linkages between segments now look quite adequate.

The dome costs, which are considerable, rise at something like the cube of the telescope length, so there is much more pressure to construct primary mirrors as fast as $F/1.75$ instead of the customary $F/3$. Such a trend will also help to shrink

the starfield onto miniature modern detectors such as CCD's, but useful angular field of view will then also shrink due to aberrations. While opticians are now confident they can devise these fast mirrors using computer-controlled polishing machines, astronomers are uneasy at the loss in field of view. It is true that big telescopes are largely used for on-axis spectroscopy, in which field-size is unimportant. However, at the conference J.R.P. Angell (Steward Observatory) demonstrated the dramatic changes we can expect from optical-fibre techniques. He used fibres for the simultaneous conduction of light from 16 widely spaced cluster galaxies down the slit of his spectrograph; thus once again highlighting the usefulness of a wide field. The battle between the big-fielders and little-fielders has scarcely begun.

The alternative to a single large instrument of revolutionary design is an array of smaller ones, adding either their light or their post-detector signal. The problems of long horizontal light beams and multiple reflections probably make a light adding design suitable only for interferometry. Any array of small instruments must reduce unit costs to the point where a very large area can be operated at a reasonable price. To reach the goal of 25 m for around \$150m the choice is either a fully instrumented 2-metre unit at \$1m, or a 4-metre at \$4m. The Kitt Peak proposal for an Advanced Technology Telescope of 2 metre size incorporates fast thin-mirror automatic setting and guiding for a projected cost around \$1.7m. How far 'mass' production could bring this down further is still uncertain. In France, A. Labeyrie (CERGA, Grasse) is operating the first of his concrete, onion-shaped 1.5-metres,

which, due to their weight do not need a dome and can cost a mere \$120,000 per square metre of collecting area.

With infra-red astronomy making great strides it will obviously be sensible if infra-red and optical astronomers can contrive to share telescopes. There was much debate on this issue, but with no clear result, mainly because infra-red astronomers see the need for at least two different kinds of instrument. There seems to be no doubt that the diameter of the image is disturbed by atmospheric 'seeing' as a function of wavelength, following a $\lambda^{-1/5}$ law as predicted by the theory of homogenous turbulence. Given a telescope of sufficient diameter to be diffraction limited, the atmosphere will allow 0.5 arc second seeing at 10 microns.

This, combined with the long awaited infra-red imaging CCD's, calls for a single aperture telescope of 10-metres or more, whose structure does not radiate significantly into the beam. On the other hand, C. Townes (Bell Laboratories) and F. Low (University of Arizona) have demonstrated phase coherence at the same wavelengths for periods of up to an hour and want coherent arrays of smaller telescopes. It would seem that infra-red astronomy will now develop along the lines of radio astronomy with a request for both phased arrays and big single dishes.

The issues and questions raised by the conference come at an appropriate time. It does now seem likely that a 25-metre equivalent mirror can be built in any one of a variety of ingenious ways. It remains to be seen which is best and cheapest but astronomers will need to unite behind a single proposal before they approach governments for a sum that is likely to be nearer two than one hundred million dollars.



100 years ago

ON THE SKIN-FURROWS OF THE HAND

A large number of prints have been taken by me from the fingers of people in Japan, and I am at present collecting others from different nationalities.

My method of observation was at first simply to examine fingers closely, to sketch the general trend of the curves as accurately as possible, recording nationality, sex, colour of eyes and hair, and securing a specimen of the latter. I passed from this to "nature-printing," as ferns are often copied.

A common slate or smooth board of any kind, or a sheet of tin, spread over very thinly and evenly with printer's ink, is all that is required. The parts of which impressions are desired are pressed down steadily and softly, and then are transferred to slightly damp

paper. I have succeeded in making very delicate impressions on glass. They are somewhat faint indeed, but would be useful for demonstrations, as details are very well shown, even down to the minute pores. By using different colours of ink useful comparisons could be made of two patterns by superposition. These might be shown by magic lantern. I have had prepared a number of outline hands with blank forms for entering such particulars of each case as many be wanted, and attach a specimen of hair for microscopic examination. Each finger-tip may best be done singly, and people are uncommonly willing to submit to the process. A little hot water and soap remove the ink.

When bloody finger-marks or impressions on clay, glass, &c., exist, they may lead to the scientific identification of criminals. Already I have had experience in two such cases, and found useful evidence from these marks. In one case greasy finger-marks revealed who had been drinking some rectified spirit. The pattern was unique, and fortunately I had previously obtained a copy of it. They agreed with microscopic fidelity. In another case sooty finger-marks of a person climbing a white wall were of great use as negative evidence. Other cases might occur in medico-legal investigations, as when the hands only of some mutilated victim were found. If previously known they would be much more

precise in value than the standard *mole* of the penny novelists.

I have heard, since coming to these general conclusions by original and patient experiment, that the Chinese criminals from early times have been made to give the impressions of their fingers, just as we make ours yield their photographs. I have not yet, however, succeeded in getting any precise or authenticated facts on that point. That the Egyptians caused their criminals to seal their confessions with their thumb-nails, just as the Japanese do now, a recent discovery proves. This is however quite a different matter, and it is curious to observe that in our country servant-girls used to stamp their sealed letters in the same way. There can be no doubt as to be advantage of having, besides their photographs, a nature-copy of the for-ever-unchangeable finger-furrows of important criminals. It need not surprise us to find that the Chinese have been before us in this as in other matters. I shall be glad to find that it is really so, as it would only serve to confirm the utility of the method, and the facts which may thus have been accumulated would be a rich anthropological mine for patient observers.

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REVIEW ARTICLE

Compound eyes: old and new optical mechanisms

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Over the last twenty years classical views of how compound eyes work optically have undergone a series of overhauls. Exner's central concept of an optically inhomogeneous lens cylinder has survived, and such devices are now made commercially. He was wrong, however, about some crustacean eyes. They produce images by a mirror mechanism that was not discovered until 1975, and which now shows promise as an optical system capable of development.

In 1891 Sigmund Exner produced a monograph¹ on the optical mechanisms of insect and crustacean eyes that was a landmark not only in biology, but also in optical theory. The problem at that time was that image formation in many animals with compound eyes was not comprehensible in terms of conventional spherical surface refraction, as in a simple lens. This is particularly true of aquatic insects and crustaceans, where there is only a negligible refractive index difference between external and internal media so that ray-bending by a curved cornea is minimal, and also in those eyes whose corneal surfaces are flat anyway—some mantids, for example². Exner's solution was ingenious and unconventional. He proposed that the optical elements of many compound eyes behaved as 'lens cylinders', that is, cylinders with a graded refractive index, densest along the axis and declining in an approximately parabolic manner towards the outside. Such structures have the property that a

non-axial ray, striking the end of the cylinder normally, will be refracted continuously towards the axis (Fig. 1*d, e*). In fact, if the gradient is right, all parallel rays will be refracted to the same point on the axis, and parallel rays at an angle to the axis will be focused in the same plane, but slightly to one side. In other words, an inhomogeneous flat-ended cylinder would behave very much like an ordinary converging lens in its image-forming properties.

Inhomogeneous lenses were not an entirely revolutionary proposal, even in Exner's day. Matthiessen³ had already proposed that the lenses of fish eyes had a variable refractive index, highest in the centre. He used this to explain why fish lenses had a much shorter focal length than a homogeneous lens with the same central refractive index (this would be about 4 radii, whereas real lenses have a focal length of about 2.55 radii, a figure that has come to be known as Matthiessen's ratio). It also

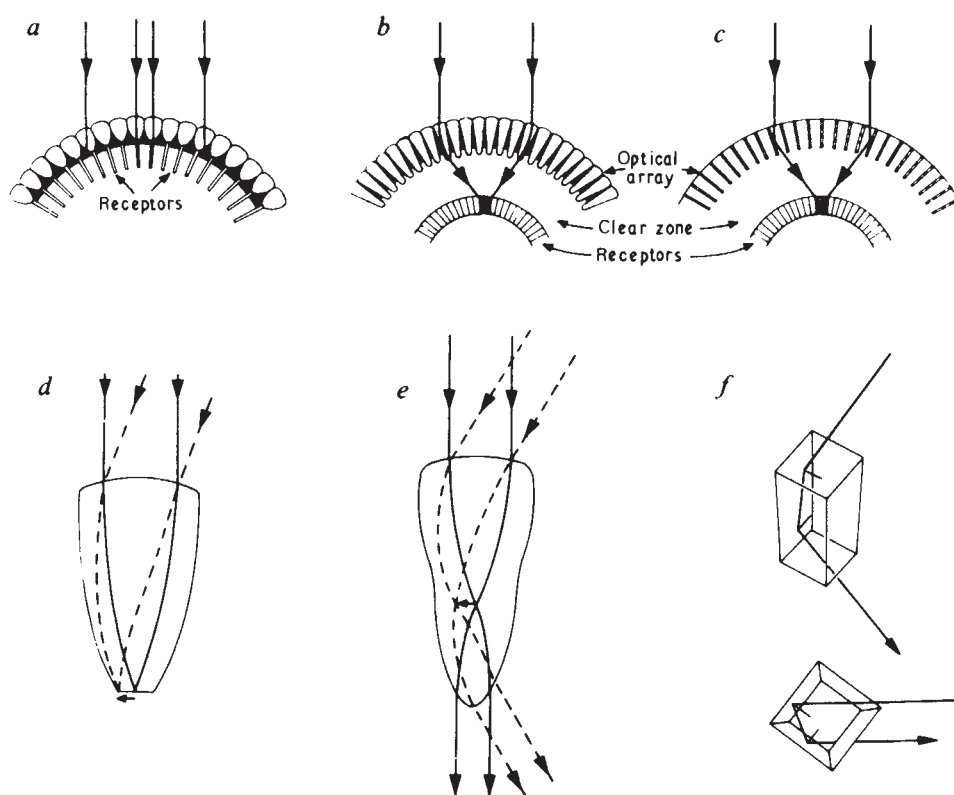


Fig. 1 Compound eye types and their optical components. *a*, Apposition eye; *b*, refracting superposition eye; *c*, reflecting superposition eye; *d*, apposition lens cylinder with focus at proximal tip; *e*, superposition (double length) lens cylinder; *f*, perspective and top view of ray path through a square cell in a reflecting superposition eye. *b, d, e* are based on Exner¹; *c* is modified from Land³⁴; *f* from Vogt³⁷.

explained why fish lenses are free of spherical aberration, which would otherwise be severe. Speculation about the properties of optically inhomogeneous media goes back to Maxwell⁴, who "stimulated apparently by the contemplation of his breakfast herring" (ref. 5), devised a fish-eye universe with the alarming property that all rays emitted from a particular point returned to it, after passing through a conjugate image⁴⁻⁶.

Much of Exner's work was based on two animals which he chose for their differences and experimental convenience. They are the king crab (*Xiphosura*) *Limulus*, and the glowworm *Lampyrus*.

Limulus

Limulus has a smooth cornea behind which is a series of ingrowths of the cuticle (Fig. 2a), each of which abuts onto a cluster of receptors. Exner found that there was an inverted image at the tip of each such ingrowth (Fig. 2c), and because of the smooth cornea concluded that this had to be produced by lens-cylinder focusing. In one particularly telling experiment he cut a parallel-sided slice through one of these structures, and showed that this still acted as a converging lens, though of longer focal length. Exner's lens-cylinder explanation of *Limulus* optics was challenged recently by Levi-Setti *et al.*⁷, who suggested that focusing might occur not by inhomogeneous refraction, but by reflection from the walls of each projection. They pointed out that the shape of the projections resembled that of an 'ideal collector', a reflecting device originally designed by Winston⁸ for the collection of faint radiation. However, such devices produce erect, not inverted images, and furthermore, the refractive index gradient in the *Limulus* cornea, measured by interference microscopy (Fig. 2b), is precisely that required for lens-cylinder optics⁹, so that Exner's mechanism survived the challenge.

Superposition images

Limulus has an apposition eye, that is, one in which each receptor cluster has its own private optics, and receives an image from a small solid angle of external space (Fig. 1a). Many nocturnal insects and crustaceans have eyes with a similar superficial appearance, but a quite different optical structure (Fig. 1b), known as superposition eyes. They are characterized anatomically by a wide zone of clear material between the superficial optics and the receptor layer, which usually forms a hemisphere with a radius of curvature about half that of the eye itself^{10,11}. One group of insects with eyes of this kind are the glowworms and fireflies (*Lampyridae*) and these have the useful feature that the optical elements are physically part of the cornea, so that by simply cleaning out the interior of the eye the

optics of the whole array can be studied. Exner did this, and found that instead of a series of inverted images at the cone tips there was just one erect image. This image was formed by the array as a whole and lay relatively deep, in approximately the position occupied by the retina. I have repeated Exner's observation (Fig. 3), as have others^{12,13}. Unfortunately, it is not easy to see an image in most insect eyes with 'clear-zones', moths for example, because the optical elements (or crystalline cones) are separate from the cornea and the array does not survive the abuse of cleaning. In choosing *Lampyrus*, and for that matter *Limulus*, Exner clearly knew what he was doing.

The explanation Exner gave for superposition image formation is shown in Fig. 1b and e. Essentially, parallel light entering many facets superimposes in the image region, and because the whole system is spherically symmetrical the overall result is an erect image. For superposition to occur it is clear that each optical element must behave as an inverter—a telescope with an overall magnification of -1. Parallel rays entering each element are redirected in such a way that the emergent beam makes the same angle with the element's axis as the entering beam, but, unlike the situation in a simple lens, the emergent beam lies on the same rather than the opposite side of the axis (Figs 1e and 3b, c).

There are two ways in which such a device could be made: as a conventional telescope made from two curved surfaces, with a focus in the middle, or from a pair of lens cylinders like those in *Limulus* but placed end to end. The former alternative is possible in theory¹ but in insects the refractive indices are too low and the curvatures inadequate for this to be the correct interpretation. Exner chose the double-length lens-cylinder explanation for *Lampyrus*. Rays are brought to a focus by the first half of the element, then unfocused and inverted by the second half. The correctness of this interpretation has now been verified for fireflies¹⁴, some other beetles¹⁵, moths¹⁶, skipper butterflies¹⁷ and euphausiid crustaceans¹⁸, in each case by examining sectioned crystalline cones using interference microscopy and confirming the existence of an appropriate refractive index gradient (Fig. 4).

Exner did, however, make one serious mistake. He generalized his mechanism to all eyes with a similar structure. Figure 1b admits of one other explanation and that is that the optical elements are simply plane mirrors (Fig. 1c). I shall return to this later.

Man-made lens cylinders

Commercial interest in inhomogeneous cylindrical lenses did not begin until the late 1960s, and the thrust was not to produce devices with compound eye-like properties, but to make optical fibres that transmitted images, as opposed to simply transmitting intensity. It is clear that an extended lens cylinder will keep imaging and re-imaging an object placed against its end at regular intervals along its length (Fig. 3d), and this ability could potentially be used to convey at least as much information per unit cross-sectional area as the alternative; this would be an expensive coherent light-guide array. The exact refractive index gradient that an image-transmitting lens cylinder should have was worked out in the mid-1950s by Fletcher, Murphy and Young¹⁹ who, incidentally, also derived the refractive index gradient for a Matthiessen fish lens. They give the following relationship between distance between foci ($2F$), axial refractive index (n_0) and refractive index (n_r) at a radial distance (r) from the axis

$$n_r = n_0 \operatorname{sech}(\pi r/2F)$$

This expression can be expanded²⁰ to give

$$n_r = n_0 \left(1 - \frac{1}{2} a^2 r^2 + \frac{5}{24} a^4 r^4 - \frac{61}{720} a^6 r^6 + \dots + \frac{E_n}{(2n)!} (ar)^{2n} \dots \right)$$

where $a = \pi/2F$, and E_n are the Euler numbers. If one ignores all terms beyond the r^2 term, the refractive index gradient

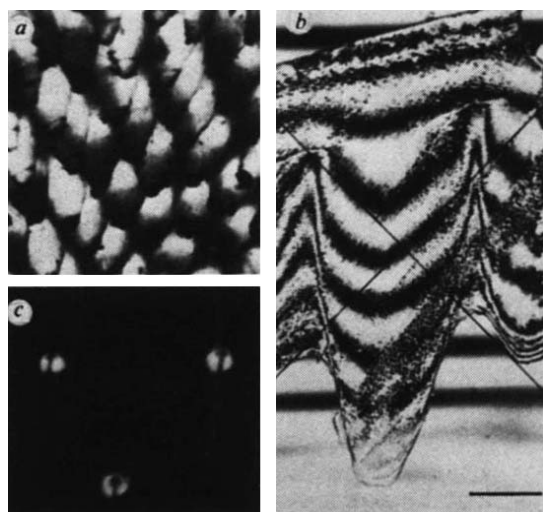


Fig. 2 Eye of *Limulus*. a, Crystalline cones projecting inwards from cornea. b, Interference micrograph of thin section of a cone. The fringe distortion is proportional to the local refractive index. Scale bar 100 μm . c, Inverted images of an arrowhead at the tips of the crystalline cones. (From Land⁹.)

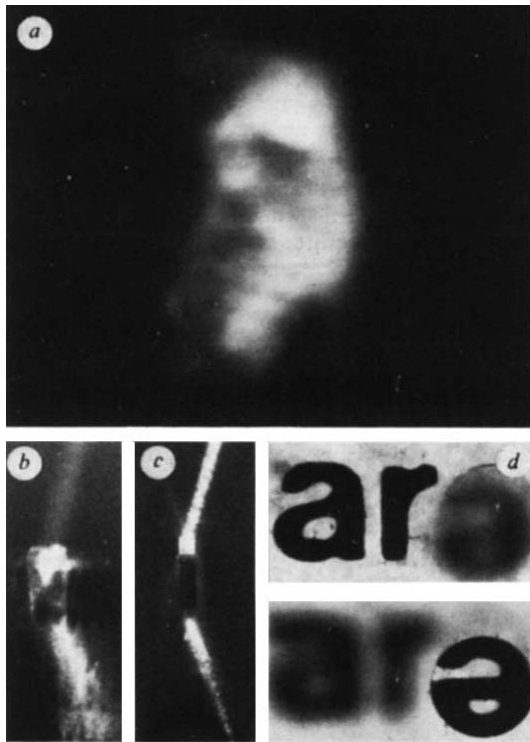


Fig. 3 Optics of refracting superposition eyes. *a*, Julia Cameron's portrait of Charles Darwin rephotographed through the cleaned cornea of a firefly. The eye was suspended in a hanging drop of water with the cornea in air. The image is erect, and the face is about 0.1 mm across. *b*, Bending of a light beam by a natural double length lens cylinder, a crystalline cone from a euphausiid crustacean. The beam is made visible with fluorescein and is 30 μm wide (from ref. 45). *c*, Light beam bent in a similar manner by a man-made SELFOC glass lens cylinder. Compare with the light paths in Fig. 1*b* and *e*. *d*, Lens cylinders also have the property of imaging an object at intervals of $2F$ (see text). The photograph shows the word 'are' with the same SELFOC as in *c* placed over the 'e'. The 'e' is re-imaged, inverted, at the upper end of the lens cylinder.

becomes a parabola, and it was this parabolic approximation that Exner's brother, Karl²¹, derived in connection with biological lens cylinders (Fig. 4). Attempts to manufacture lens cylinders have also been made using diffusion processes which give rise naturally to parabolic gradients. In the 1960s attempts were made to use gas mixtures to produce lenses and waveguides²². Then in 1968 a glass lens cylinder (SELFOC) was produced and patented in Japan. It was made by heating a thallium-rich flint glass in a molten salt (KNO_3 , at 500 °C) which leached out and replaced the heavy metal ions from the outside, thereby reducing the refractive index from 1.542 in the centre to 1.517 at the periphery^{23,24}.

More recently, plastic lens cylinders have been made by heating a high refractive index rod of soft polymerized plastic (diallyl isophthalate, $n = 1.57$) in a bath containing the monomer of a low index plastic (methylmethacrylate, $n = 1.49$)^{25,26}. This gives a usable refractive index range that is similar to that of the glass lenses.

The future of man-made lens cylinders is unclear. They have good imaging properties over short distances—through one or two serial images (Fig. 3*d*)—but then aberrations of various kinds tend to degrade the image. The higher-order terms in the expansion for n , begin to become important, as does the fact that non-paraxial rays require a somewhat different index gradient from paraxial rays²⁰. Chromatic aberration also accumulates, although this can be offset to some degree by a suitable choice of materials²⁶. Given these problems, as well as those of manufacture, it is likely that aligned fibre bundles offer better prospects for long-distance image transmission than focusing lens cylinders. There should, however, be many other applications for versatile lens-like systems of very small dimensions.

Problems, and the light-guide heresy

During the 1960s, when commercial lens cylinders were at last being developed, their biological counterparts almost suffered extinction, as the following quotations show. Of lens cylinders: "It seems very doubtful if 'lens cylinder' properties will ever be found in a compound eye and therefore it would probably be better to discard the idea." (ref. 12). And of superposition optics: "There is no reason to suppose that this is true for any compound eye, and there are several reasons, given above, why the superposition image of the firefly is no more than an artifact of the cleaned retina." (ref. 27).

The revolt against Exner's principal ideas began with some careful observations by Kuiper¹², showing that in certain crustacean eyes there are no important refractive index variations in the crystalline cones, a conclusion subsequently verified by interference microscopy^{28,29}. This would seem to rule out both lens-cylinder optics and superposition imagery. In their place Kuiper suggested that the important optical elements in this kind of eye were tracts or threads which crossed the clear zone, conveying images from lens systems in the region of the cornea to the deep-lying receptors. In support of this idea, he pointed out that these higher-index light-guide-like structures often became lined with pigment in the light-adapted eye, the function of which was to 'bleed' light out of the light guides by bringing high-refractive index material into contact with their sides. Effectively, Kuiper was suggesting that superposition eyes, in crustaceans at least, were really apposition eyes with a 'longitudinal pupil' mechanism for protecting the eye against high light intensities, rather than a superposition mechanism for increasing sensitivities at low intensities. Further support for this idea seemed to come from an interference microscope study by Allen³⁰, who claimed that in the hornworm moth the crystalline cones had a refractive index that was constant to less than 1%, and that the threads traversing the clear zone had an index of 1.523 (which would make them solid). These observations have not been confirmed, and indeed all subsequent work on insect superposition eyes¹⁴⁻¹⁷, including moths, has led to opposite conclusions¹¹.

The present position is a satisfactory compromise. Most, if not all, insect eyes with clear zones do behave as proper Exner-type superposition eyes in the dark, with the threads that cross the clear zone interfering only minimally with the ray paths that contribute to the focused image. However, the effect of light adaptation is to cause pigment movement into the clear zone,

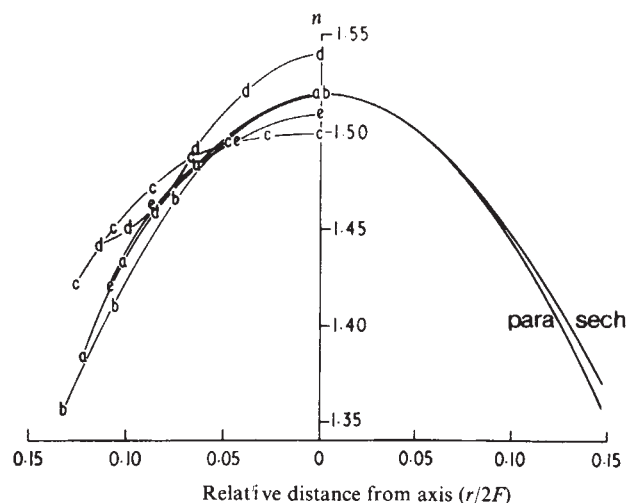


Fig. 4 Comparison of theoretical (right) and measured (left) refractive index distributions through crystalline cones. The theoretical curves show the difference between the Fletcher *et al.* equation¹⁹ and Exner's parabolic approximation. Measured data are from: *a*, a euphausiid¹⁸; *b*, a firefly¹⁴; *c*, a moth¹⁶; *d*, a skipper butterfly¹⁷; *e*, *Limulus*⁹. The gradients have been normalized by taking the cone length as $2F$ for superposition eyes (*a-d*), and F in the case of the apposition eye of *Limulus* (*e*).

confining image-forming rays to the tract of thread joining each facet to its corresponding receptor bundle. Kuiper's light-bleeding mechanism, as well as simple pigment screening, probably both occur^{31,32} and the net effect is that eyes with superposition optics at night have apposition optics by day. In moths one can watch this change happening. When suddenly illuminated in the dark, there is a large patch of 'glow' or eye-shine corresponding to the whole pupil of the superposition system, illuminated from within by light reflected from a tapetum behind the receptors (Fig. 5d). Exposure to light causes this patch to shrink to not much more than one facet, as pigment migrates inward and intercepts the oblique rays that would go to form the superposition image³².

There remains, however, the problem that started the controversy. What happens in crustacean eyes without lens cylinders?

Reflecting superposition optics

Klaus Vogt provided the answer in a short note on crayfish eyes³³. "Rays from an object point entering through different facets are superimposed not by refracting systems as in other superposition eyes but by a radial arrangement of orthogonal reflecting planes which are formed by the sides of the crystalline cones and purine layers surrounding them." Without being aware of Vogt's paper I reached the same conclusion about the eyes of a deep-sea shrimp³⁴. If one looks at Exner's original figure^{1,11} it is clear that the elements he drew could equally well be replaced by plane mirrors (Fig. 1b, c), and this is precisely what happens in certain crustacea, specifically the long-bodied decapods (shrimps, prawns, crayfish and lobsters, but not the true crabs). The structure of their eyes is very much like that of insect superposition eyes—a peripheral array of optical elements, a wide clear zone and a deep-lying hemispherical retina. They ought, anatomically, to be superposition eyes. The crucial difference is that the 'crystalline cones' are not crystalline—they are jelly-like with a refractive index of about 1.42—and neither are they conical. They are square in surface view (Fig. 5a, d), as shown perfectly accurately in Grenacher's plate of 1879 (ref. 35) (Fig. 5b).

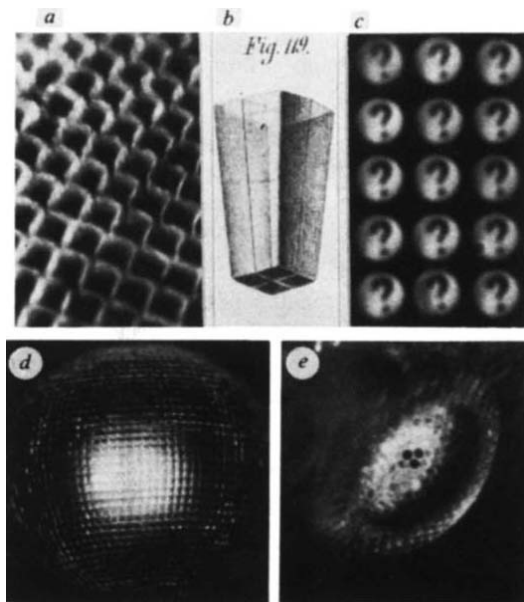


Fig. 5 Reflecting superposition optics. *a*, Surface of a crayfish eye showing the square array of mirrors; *b*, Grenacher's drawing³⁵ of an optical element from a shrimp; *c*, images formed by lenses in the cuticle of a crayfish eye, which prefocus the beams entering the mirror boxes⁴¹; *d*, adult eye of *Palaemonetes varians*, fully dark adapted, showing square symmetry and patch of eye-shine corresponding to the superposition pupil; *e*, 1st instar larva of *Palaemon serratus*, showing hexagonal symmetry and a small black pseudo-pupil characteristic of apposition eyes.

There is an elegant reason that eyes that use mirrors must have square facets. Figure 1c shows a two-dimensional diagram of an image-forming mirror array, but the question is: of what three-dimensional array would this be a section? One possibility is that it is a section of an axially symmetrical arrangement of tilted circular strips (Fig. 6a). Clearly, this will form an image of a distant point source located on the axis of the structure, but equally clearly it will not form an image of light originating from any other direction: the strips will not so much focus light as get in the way of it. What is required is an array that will behave like the strip arrangement, but for all directions. An array of square corners will do this. The reason is that a pair of mirrors at right angles to each other behave as though they were a single mirror that is always normal to the plane of the incident ray (Figs 1f and 6b): the complete angle through which the ray is reflected must add up to 180°. (One occasionally encounters corner mirrors in clothes shops—it is impossible to escape from one's image by moving around them.) Thus, provided most light entering the eye encounters two faces of each mirror box, all ray paths to the focus will be essentially identical to those of the two-dimensional diagram^{29,36,37}. For a large-aperture eye like that of a crayfish each mirror box should be about twice as deep as it is wide, for the double reflection conditions to be met.

Not all eyes of the reflecting superposition type actually have mirrors. In the shrimp *Palaemonetes*, for example, reflection occurs at the faces of the mirror box by total internal reflection. With a refractive index of 1.41 inside the box, and a fluid outside with an index of about 1.33, the critical angle for total internal reflection is 71°, that is, rays making angles of up to 19° with the box wall will be reflected. This restricts the eye's effective pupil to about one-third of the eye diameter. To increase this the boxes are lined with a specular mirror in both crayfish and deep-sea shrimp. The mirrors are composed of a three-layer sandwich of one-quarter wavelength-thick plates alternating with tissue fluid³⁷, which is a common way for natural mirrors to be produced^{38,39}. The nature of the material of the plates is not known in the crayfish, but in a penaeid shrimp it is the pteridine isoxanthopterin⁴⁰.

A final refinement of the crayfish optical mechanism was found recently by Bryceson⁴¹. She noticed that each facet of the cleaned cornea is actually a long focus lens, with a focal length in water approximately equal to the distance from cornea to receptors (Fig. 5c). This has two implications. It means that the pencil of light passing through each mirror box is prefocused, so that it reaches the receptors as a fine beam, thereby improving image resolution. It also means that when the eye is in the light-adapted condition and each receptor only receives light from its 'own' facet⁴², the focusing ensures a resolved image on that receptor and hence a narrow field of view. Interestingly, Kuiper had noticed inverted images in his original study¹².

Phylogeny and ontogeny

Mirror boxes with a hexagonal cross-section do not reflect light as shown in Fig. 1f and 6b, and so cannot be used in reflecting superposition eyes. Therefore, hexagonally faceted eyes do not use this mechanism, whereas square-faceted eyes probably do. Corneal geometry can thus be used as a reliable indication of an eye's optical type, and, since this is likely to be an evolutionary conservative character, of its ancestry as well^{43,44}.

An examination of the higher crustacea shows that square-faceted eyes are found only in the Decapoda, and there only in the long-bodied forms. The crabs (*Brachyura*) and hermit-crabs (*Anomura*), but interestingly not the squat-lobsters (also *Anomura*), have hexagonally faceted eyes of the apposition type, without a clear zone^{43,44}. Equally interesting is the fact that the euphausiids, the shrimp-like krill usually grouped with the decapods in the super-order Eucarida, have both hexagonal facets and a clear zone. These eyes are very similar in their construction to the refracting superposition eyes of moths, and indeed their bullet-like crystalline cones bend light in the same way as in insect superposition eyes⁴⁵ and have a lens cylinder-

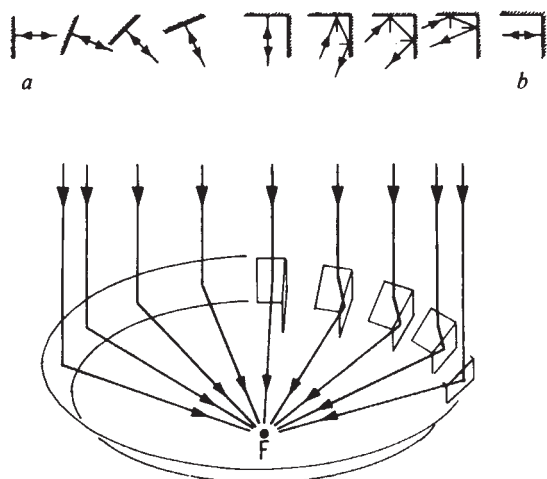


Fig. 6 A superposition focus can be formed either by set of tilted circular strips of mirror (a, left) or by a surface made of corner reflectors (b, right). Corner reflectors behave as though they are single mirrors at right angles to the plane of the incident ray (inserts above) so that the ray paths are almost the same as for the strips.

like refractive index profile¹⁸. The mysids (Peracarida), which look very much like euphausiids but differ from them in having a brood-pouch, also have refracting superposition eyes, although other peracaridans have apposition eyes. In the last century the euphausiids and mysids were grouped together as the Schizopoda, and my guess is that that grouping more accurately reflected their evolutionary affinity.

The decapods with reflecting superposition eyes do not start life that way (Fig. 5e). In their larval stages they have hexagonally faceted eyes with a typical apposition structure. In *Palaemonetes* it is not until about moult 15, when the animal is almost adult, that the facets square off and the superposition mechanism comes into operation. Presumably, the apposition type of eye is adequate for the upper ocean where the planktonic larvae live, just as it is in the light-adapted adults, but the deeper, dimmer waters the adults inhabit on maturity make it advantageous to use superposition optics, with their much greater light-gathering power. Larval crabs have eyes just like larval shrimps: the main developmental difference is that adult crab eyes retain the larval optical design. Again this makes sense, since most of them are shallow water or even terrestrial forms and would not need the superposition mechanism. It is as though the crab eye is derived from the crayfish eye by neoteny, the final developmental stage having been omitted.

This is somewhat perplexing for taxonomists. Eye structure links the mysids and euphausiids, which contemporary taxonomy does not. The reflecting superposition mechanism links all the long-bodied decapods, which are currently split between the Natantia and Reptantia⁴⁶ or the Dendrobranchiata and Pleocyemata⁴⁷. It splits the galatheids (squat lobsters) from the pagurids (hermit crabs) and so divides the Anomura, and it

suggests that the euphausiids and decapods are unrelated. Any one character is perhaps as fallible as any other as an evolutionary guide, but at least for eyes one can make the claim that once a design that works well has evolved it is most unlikely to be abandoned for an equally complicated but functionally equivalent one, as, for example, reflecting versus refracting superposition optics.

Although the decapods are alone among crustacea in possessing square-faceted eyes, there may be one possible parallel in insects. Horridge and Maclean⁴⁸ have described the eye of the Australian mayfly *Atalophlebia*, which has entirely square facets in its dorsal eye (in males the eyes are divided), square homogeneous crystalline cones and a wide clear zone. The optics are not well understood, but this ought to be a reflecting superposition eye. By contrast, the common European mayflies (*Cloeon*) have hexagonal facets and presumably refracting superposition optics. The Ephemeroptera are again a taxonomically difficult group, and it will be interesting to see whether eye structure is helpful here as well.

X-ray telescopes and other applications

Both types of superposition eye represent image-forming systems equivalent in many ways to conventional lenses and mirrors, but which have so far found few applications in optical technology. It is almost true to say that they have not properly been invented. In the case of refracting superposition devices it is clear that the task of making a large number of appropriate lens cylinders and aligning them accurately probably is not worth the effort, but for a reflecting system this is not necessarily true. One could imagine the construction of a reflecting array from strips of sheet metal, cut so as to interlock, or from a casting that was subsequently plated (there is a problem in trying to cover a sphere with squares—basically it cannot be done—but both geographers and lobsters have found ways round it). Such structures could have wide apertures, like crustacean eyes, and serve in applications like solar collectors, where condensing power rather than resolution is at a premium, or as emitters they could be useful for making large collimators, lighthouse lenses for example.

If the pupil is restricted, much higher resolution can be achieved, and it is this form that has produced the only important application so far, as an X-ray telescope^{49,50}. X rays cannot be focused by refraction or by reflection at near normal incidence, which has meant that existing X-ray telescopes are all based on grazing incidence optics utilizing nested surfaces. Their design is basically similar to the tilted strips in Fig. 6a, and they suffer from the same drawback, a very narrow field of view. Angel⁴⁹ realized that the use of square cells as reflectors eliminates this problem, and has designed a telescope with a spatial resolution of 30 arc s. This degree of resolution is achieved by making the reflecting cells very long—about 100 times their width. Because they are reflecting devices, square-cell surfaces are usable over large parts of the electromagnetic spectrum, and provided the manufacturing problems can be overcome they should have a wide range of uses.

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ARTICLES

Guanine nucleotide-binding and autophosphorylating activities associated with the p21^{src} protein of Harvey murine sarcoma virus

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The purified p21^{src} protein of Harvey sarcoma virus shows a guanine nucleotide-binding activity and, in addition, at elevated temperature an autophosphorylating activity at a threonine residue using as phosphoryl donor GTP or dGTP but not ATP or dATP. These biochemical activities are unique among those associated with transforming proteins of RNA-containing or DNA-containing tumour viruses.

HARVEY MURINE SARCOMA VIRUS (Ha-MSV) is a replication-defective retrovirus with the potential to transform fibroblasts in cell culture and to induce solid tumours and erythroleukaemia in animals. It is a recombinant virus between genetic sequences of a helper-independent mouse type-C virus at both ends of the genomic viral RNA and inserted rat nucleic acid sequences^{1–3}. Using antisera from rats bearing Ha-MSV-induced tumours, we have identified a 21,000-molecular weight (MW) protein (p21) coded for by the rat insert sequences in Harvey virus-transformed cells and in cell-free translation products of Ha-MSV virion RNA⁴. One form of p21 is phosphorylated in virus-transformed cells. Two major lines of evidence have led us to conclude that one of the forms of p21 is the transforming *src* gene product of Ha-MSV. First, the p21 encoded by a mutant (ts371) of the closely related Kirsten murine sarcoma virus (Ki-MSV), temperature-sensitive for the maintenance of transformation, is much more thermally labile than the p21 of the wild-type Kirsten virus⁵. Second, through transfection studies of the molecularly cloned Ha-MSV proviral DNA, its cloned subgenomic fragments and a series of Ha-MSV deletion-insertion mutants, the transforming *src* gene and the p21 encoding region have been localized to the 1,100-nucleotide rat sequences closest to the 5' end of the viral genome^{6–8}. The gene coding for an endogenous p21 also seems to be present in uninfected normal cells derived from a variety of vertebrate species. The antisera containing antibodies to the Ha-MSV p21 precipitated a smaller quantity of the 21,000-MW cellular homologue protein which is structurally and antigenically related to the Ha-MSV p21 and is itself highly conserved throughout evolution⁹. The major problem is, through what kind of molecular mechanism the p21 *src* transforms normal cells. Over 95% of the intracellular p21^{src} of a Ha-MSV-transformed MDCK dog cell has been localized to the inner surface of

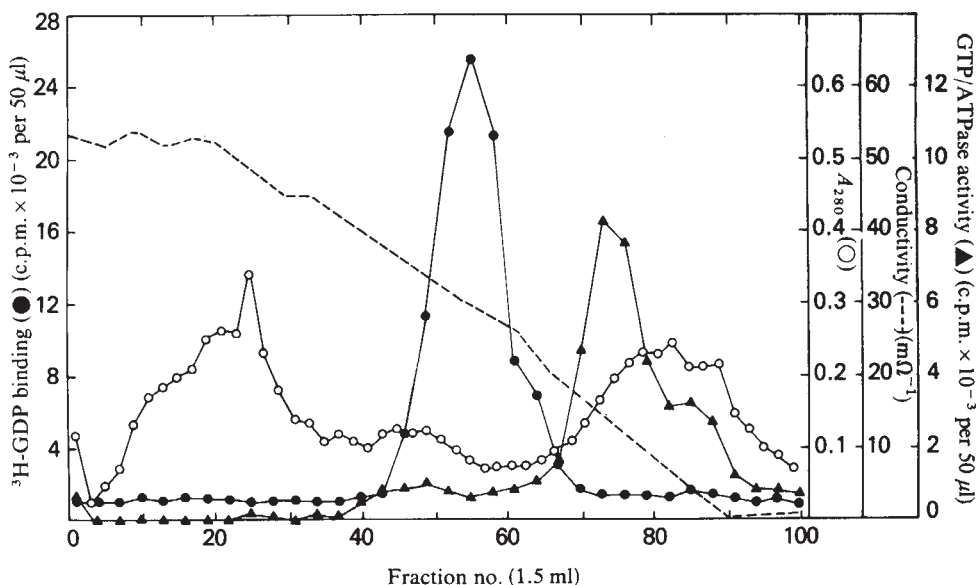
the plasma membrane by electron microscopic immunocytochemistry suggesting the cellular membrane to be a major site of action of this *src* protein¹⁰. Perhaps the most interesting property of the p21^{src} is its strong affinity for guanine nucleotides, implying that the transforming function of the p21^{src} may involve the metabolism of guanine nucleotides¹¹.

The p60^{src} gene product of Rous sarcoma virus (RSV)^{12–15} and the T antigens of adenovirus¹⁶ and polyoma virus¹⁷ are associated in immunoprecipitates with an ATP-protein kinase activity which phosphorylates the immunoglobulin heavy chain. A tyrosine-specific phosphorylating activity has been found in immunoprecipitates of polyoma T antigens¹⁸, Abelson murine leukaemia virus (MuLV) p120 (ref. 19) and RSV p60^{src} (ref. 20). We report here a unique GTP-specific enzymatic activity of the partially purified Ha-MSV *src* protein which in solution without immunoprecipitation autophosphorylates the p21^{src} with [γ -³²P]GTP but not with [γ -³²P]ATP as phosphoryl donor. This enzymatic activity is clearly distinct from that of most of the other cellular protein kinases.

Purification of the p21 from NIH 3T3 cells transformed by Ha-MSV

Using antisera containing antibodies to the p21, a ³H-GDP-binding assay specific to p21 has been developed¹¹. Cellular extracts are incubated with ³H-GDP and the antigen-antibody complexes precipitated by an absorbent, formaldehyde-fixed *Staphylococcus aureus*-containing protein A or protein A-Sepharose (Pharmacia). p21 in the immunoprecipitates is quantified by the ³H-GDP bound to the p21 protein. This rapid assay allows purification of p21 from unlabelled cell extracts on a large scale. To preserve any enzymatic activity associated with p21, purification procedures used for most enzymes in

Fig. 1 Purification of the p21 by phenyl-Sepharose chromatography. 20 ml of hydroxyapatite-purified p21 (18 mg) in 0.09 M sodium phosphate, pH 6.8, was adjusted by adding 3.5 M ammonium sulphate to a final concentration of 0.8 M. It was loaded slowly on a small phenyl-Sepharose column (5 ml bed volume, Pharmacia) equilibrated with a starting buffer containing 0.8 M ammonium sulphate, 0.01 M sodium phosphate, pH 6.8, and 1 mM DTT at 4 °C. After washing in the sample with an additional 10 ml of the starting buffer, the column was eluted with 80 ml of a linearly decreasing ammonium sulphate gradient from 0.8 to 0 M in the same buffer at a flow rate of 0.2 ml min⁻¹. After completion of the gradient, the column was further eluted with 40 ml of the same buffer without ammonium sulphate and 1.5-ml fractions were collected. Immediately after measuring A_{280} and conductivity of the fractions, the fractions were dialysed against 50% glycerol in 0.01 M sodium phosphate, pH 6.8, and 1 mM DTT. The samples were stored at -20 °C, and no loss of p21 enzymatic activity was observed in this storage condition for at least 2 months. The p21 in column fractions was detected by a p21-specific ³H-GDP binding assay as described in Table 1 using anti-p21 rat antiserum. In those conditions of assay, 7,300 c.p.m. represent 1 pmol of ³H-GDP bound. As an illustration of the resolving power of the hydrophobic column, a cellular ATP/GTPase activity was also assayed by incubation with [γ -³²P]GTP across the gradient³⁴. The ³²P-orthophosphate released by the enzyme reaction was extracted into the benzene/isobutanol organic phase as a silicotungstic-phosphomolybdate complex. In those assay conditions, 100 c.p.m. represents 1 pmol P_i produced in 20 min at 37 °C. ³H-GDP-binding activity of the p21 (●); A_{280} (○); conductivity (---); ATP/GTPase activity (▲).



nondenaturing conditions were used. The membrane-bound p21 was solubilized by homogenizing cells in a buffer containing 1% Triton X-100. After high-speed centrifugation to remove nuclei and insoluble cell debris, the cell lysate was chromatographed on a hydroxyapatite column and eluted with a step-gradient of increasing sodium phosphate concentration. Most of the p21 was recovered in a fraction eluted between 0.005 and 0.09 M sodium phosphate. Essentially no detectable p21 was present in the flow-through material or fractions eluted with higher phosphate concentrations of 0.15 and 0.3 M. The specific binding activity of ³H-GDP was increased 17-fold from the high-speed supernatant with a recovery of 172% total activity (Table 1). This increased activity was due to removal of substances interfering with the binding assay in crude extracts.

Hydroxyapatite chromatography is a valuable step in the purification scheme; it removes Triton X-100 and most other substances, such as lipid and nucleic acid. As p21 is mostly bound to membrane, chromatography based on hydrophobic interactions may be of particular value. Figure 1 shows the purification of p21 on a phenyl-Sepharose column. The hydroxyapatite fraction was applied to the column in 0.8 M

ammonium sulphate and eluted with a decreasing gradient of ammonium sulphate which loosened the hydrophobic interaction. The p21 emerged as a single sharp peak at ~0.4 M ammonium sulphate at the minimum of the A_{280} profile. A cellular ATPase activity was detected at 0.2 M ammonium sulphate and was clearly separated from p21. As Table 1 shows, this step purified the specific GDP-binding activity of p21 by 16-fold. The overall purification from the high-speed supernatant of cell lysate was 265-fold with recovery of 93% total binding activity. To verify more directly that the purified GDP-binding activity represented the activity associated with the p21 protein, cellular proteins labelled in tissue culture with ³⁵S-methionine were purified by hydroxyapatite and phenyl-Sepharose chromatography. Both phosphorylated and non-phosphorylated forms of the p21 doublet were visualized by immunoprecipitation with antisera and SDS-gel electrophoresis from fractions eluted at 0.09 M sodium phosphate in the hydroxyapatite column and at 0.4 M ammonium sulphate in the phenyl-Sepharose column. The chromatographic profiles of the ³⁵S-methionine-labelled p21 on both columns were identical to that detected by the GDP-binding assay.

Table 1 Summary of p21 purification

Step	Total protein (mg)	Total GDP-binding activity (pmol GDP)	Specific binding activity (pmol mg ⁻¹)	Yield (%)	Step purification	Total purification
Total homogenate (50 g cells)	4,292	—	—	—	—	—
1. High-speed supernatant	2,523	2,291	0.9	100	1×	1×
2. Hydroxyapatite (0.09 M PO ₄ fraction)	261	3,944	15.1	172	17×	17×
3. Phenyl-Sepharose (fractions 52–58)	9	2,131	241.0	93	16×	265×

All the purification procedures were performed at 4 °C. Approximately 50 g of Ha-MSV-transformed NIH 3T3 cells were lysed in 4 vol of lysis buffer containing 1% Triton X-100, 0.1 M NaCl, 0.005 M MgCl₂, 0.02 M Tris-HCl, pH 7.4, and 0.1 mM dithiothreitol (DTT). Cell lysis was assisted by two passages through an 18-gauge needle in a syringe. This is referred to as the total homogenate which was centrifuged at 150,000g for 30 min, and the pellets discarded. This fraction is the high-speed supernatant. For hydroxyapatite chromatography, the high-speed supernatant was dialysed overnight against the starting buffer (0.005 M sodium phosphate, pH 6.8, and 0.1 mM DTT) and was then loaded on a hydroxyapatite column (5 × 25 cm, Bio-Rad). The flow-through material was washed with the starting buffer until the A_{280} returned to the basal level. The fraction containing p21 was then batch-eluted with 0.09 M sodium phosphate, pH 6.8, and 0.1 mM DTT. The p21 was detected by a ³H-GDP-binding assay¹¹. Briefly, various amounts of column fractions were incubated at 4 °C with 10⁻⁵ M of ³H-GDP (10 Ci mmol⁻¹) for 10 min in a final volume of 0.3 ml (1% Triton, 0.2 M NaCl, 0.005 M MgCl₂ and 0.02 M Tris-HCl, pH 7.4). Antisera or normal sera (7 μl) as controls were then added. After 90 min, the antigen-antibody complexes with bound ³H-GDP were precipitated with 50 μl 10% (v/v) formaldehyde-fixed *S. aureus*. After several washings, the precipitates were dissolved by boiling in 0.30 ml of 2% SDS, 28 mM β-mercaptoethanol, 100 mM Tris-HCl, pH 7.8, 40% glycerol. ³H-GDP dissolved in the supernatant was then counted. The ³H-GDP binding activity of p21 was determined from the linear portion of the binding versus protein curves. The protein concentration was determined by the method of Lowry³³. The hydroxyapatite fraction was stored in small aliquots at -170 °C, and subjected to only one cycle of freeze-thawing. Portions of this fraction were further purified by phenyl-Sepharose column detailed in Fig. 1 legend. The activity and yield shown above represent the total activity from the original 50-g cell pack.

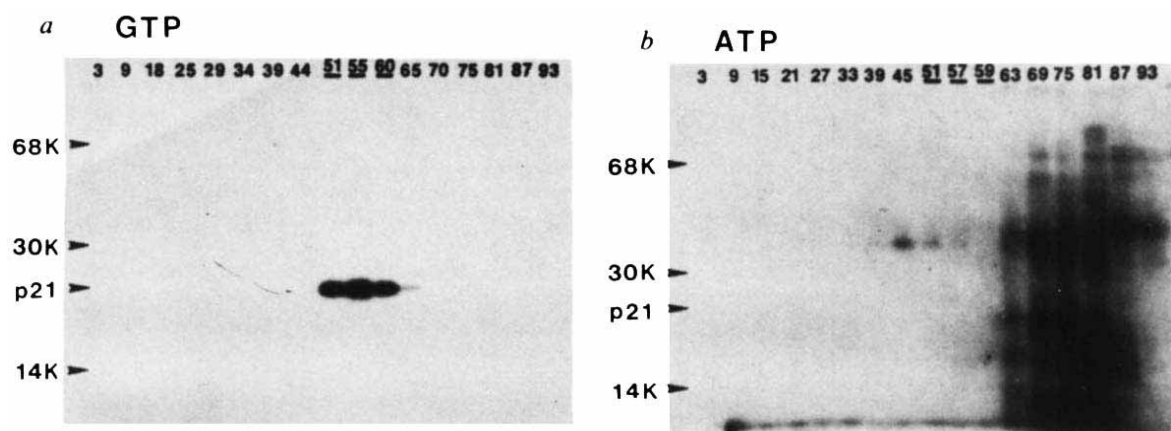


Fig. 2 Autophosphorylation activity of p21 and nucleotide-substrate specificity. Phenyl-Sepharose column fractions (indicated by the same fraction numbers as in Fig. 1) were assayed for phosphotransferase activities using various [γ - 32 P]-labelled nucleotide triphosphates. The p21-peak fractions as detected by 3 H-GDP binding assay were shown by the underlined fraction numbers. Column fractions (40 μ l) containing approximately 6 μ g protein in the peak were incubated at 30 $^{\circ}$ C for 40 min in a 100- μ l reaction mixture containing 0.01 M sodium phosphate, pH 6.8, 5 mM MgCl_2 , 1 mM DTT, protein acceptors (5 mg ml^{-1}) as indicated, and various [γ - 32 P]nucleotide triphosphates (~ 15 Ci mmol^{-1}) at a final concentration of ~ 4.7 μ M. The exact condition for each experiment is indicated below. Except for the immunoprecipitation experiment (e), at the end of incubation the reaction was stopped by the addition of 0.5 ml cold 10% TCA. After standing at 4 $^{\circ}$ C for 60 min, the precipitated protein was pelleted by 10 min centrifugation in the Eppendorf microfuge. After being washed once more with 1 ml of 10% TCA at 4 $^{\circ}$ C, the pellets were dissolved by incubating at 95 $^{\circ}$ C for 3 min in 50 μ l of 2% SDS, 0.2 M Tris-HCl, pH 8.0, 40% glycerol, 28 mM β -mercaptoethanol and 0.002% bromophenol blue. Proteins were separated by a 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described⁴. The dried gels were autoradiographed for 18–72 h. The 14 C-labelled protein markers are bovine serum albumin (68K), carbonic anhydrase (30K) and lysozyme (14K). a, Column fractions were incubated with 2.7 μ M of [γ - 32 P]GTP (19 Ci mmol^{-1} , Amersham) in the presence of phosvitin (5 mg ml^{-1} , Sigma) which was visualized as a 40K band in Coomassie blue-stained gel. b, Column fractions were incubated with 5.6 μ M of [γ - 32 P]ATP (18 Ci mmol^{-1} , Amersham) in the presence of phosvitin (5 mg ml^{-1}). c, Column fractions were incubated with 1.8 μ M of [γ - 32 P]dGTP (25 Ci mmol^{-1} , ICN) in the presence of casein (5 mg ml^{-1} , Sigma) which revealed itself in the stained gel as a 25K band above the p21. d, Column fractions were incubated with 4.3 μ M of [γ - 32 P]dATP (10 Ci mmol^{-1} , ICN) in the presence of casein (5 mg ml^{-1} , Sigma). e, Column fractions were incubated with 9.3 μ M of [γ - 32 P]GTP (5 Ci mmol^{-1} , Amersham). After incubation, the p21 was immunoprecipitated by 7 μ l of anti-p21 antiserum from tumour-bearing rats by a procedure previously described⁴. The immunoprecipitate was analysed by SDS-PAGE.

Nucleotide binding specificity of the purified p21

We have shown previously¹¹ that in crude cell lysates, the 3 H-GDP binding of p21 can be effectively inhibited by 20-fold excess of unlabelled GDP, GTP, dGTP, ppGpp, p[CH₂]ppG and to a lesser extent UTP but not by other nucleotide triphosphates, diphosphates, monophosphates and 3',5'-cyclic phosphates. In crude extracts, the thermolability of the p21 encoded by the temperature-sensitive mutant of the closely related Ki-MSV can also be stabilized against thermal inactivation by 0.1 or 1 mM of GDP, GTP or dGTP. Using the purified p21 we examined the specificity by direct binding of 3 H-labelled nucleotides. As shown in Table 2, a constant amount of the p21 purified by phenyl-Sepharose was incubated with 4–12 μ M of 3 H-labelled nucleotides; the bound nucleotides were immunoprecipitated as a complex with p21 by the antiserum. Only GTP, dGTP, GDP and to a lesser extent dATP demonstrate significant binding with normal serum. Nucleotides bound were approximately the same for GTP, GDP and dGTP, whereas dATP was only 24% of dGTP. Note that, in contrast to GTP, ATP gives no appreciable binding.

The nucleotide concentration for half-maximal binding was determined using p21 purified by phenyl-Sepharose in the

3 H-nucleotide concentration range from 2×10^{-9} to 10^{-7} M. In Mg^{2+} -containing buffer (5 mM MgCl_2 , 0.2 M NaCl, 0.02 M Tris-HCl, pH 7.4), the concentration for half-maximal binding for GTP was observed at 8×10^{-9} M, for GDP at 7×10^{-9} M, and for dGTP at 9×10^{-9} M. The maximum amount of nucleotide bound was approximately the same for each of these three nucleotides. Although dATP gave appreciable binding, the concentration for half-maximal binding was $\sim 5,000$ times higher than that for dGTP. Contamination by dGTP of less than 0.02% could account for the dATP binding observed here. In buffers containing 5 mM EDTA and no added Mg^{2+} the same maximal extent of binding was observed but 100-fold higher concentrations of GTP were required for half-maximal binding.

These binding studies indicate that p21 is a guanine nucleotide-binding protein with strong affinity towards GTP, GDP and dGTP. Nucleotides of other bases and all monophosphates demonstrate much less significant binding to p21.

GTP-specific autophosphorylation of p21

The p21 immunoprecipitated from 35 S-methionine-labelled lysates of virus-transformed cells migrated as a doublet band in SDS-polyacrylamide gel electrophoresis; the lower band was a non-phosphorylated form of the p21, and the upper band was

labelled with ^{32}P -orthophosphate *in vivo*. The high resolving power of the phenyl-Sepharose chromatography allowed us to study the possible enzymatic activities associated with p21. The guanine nucleotide-binding properties of p21 suggest that it may have enzymatic activity involving these nucleotide triphosphates. Small aliquots of the column fractions were incubated with various $[\gamma\text{-}^{32}\text{P}]$ -labelled nucleotide triphosphates and the total proteins phosphorylated *in vitro* were then examined by SDS-polyacrylamide gels without prior immunoprecipitation. The results are shown in Fig. 2. Using $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ as a substrate (Fig. 2a), the only major protein phosphorylated across the column fractions is the $\sim 21\text{K}$ band. This 21K band is most intense at the underlined fractions of numbers 51, 55 and 60, and coincides precisely with the p21 peak as determined by ^3H -GDP-binding assay seen in Fig. 1. That the 21K band is indeed the p21 protein is demonstrated by its immunoprecipitation with antisera containing antibodies to p21 (Fig. 2e) and its co-migration with the phosphorylated *in vivo* upper band of the p21 in SDS-gel electrophoresis (data not shown). It will be important to determine which form of the p21, the phosphorylated or non-phosphorylated, becomes auto-phosphorylated. In contrast to the results obtained with GTP, when $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was used as phosphoryl donor, no p21 was phosphorylated at the peak fractions 51, 57 and 59 (Fig. 2b). The addition of $10\text{ }\mu\text{M}$ cyclic AMP had no effect on these patterns of phosphorylation. However, with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, extensive phosphorylation of various proteins are seen especially in fractions from 63 to 93, corresponding to the A_{280} peak seen in Fig. 1. This intense ATP-dependent protein kinase activity was well separated from the p21 peak in the phenyl-Sepharose chromatography. In Fig. 2c, it is seen that phosphorylation of p21 occurs at the same peak fractions by using $[\gamma\text{-}^{32}\text{P}]\text{dGTP}$ as donor. The dGTP-dependent activity is similar to the GTP activity. The dATP-dependent reaction is also similar to ATP (Fig. 2d), and again no p21 phosphorylation is seen at the peak fractions. The lack of phosphorylation by $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ is not due to the phosphatase or pyrophosphatase activity in the preparation which selectively degrades ATP: most of the $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ or $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ remained intact after incubation with p21 for 80 min (Fig. 3). Compared with the control with no p21, very little ^{32}P -orthophosphate or ^{32}P -pyrophosphate was formed by incubation with the p21 preparation. Therefore, the phosphorylation reaction is very specific to GTP and dGTP and parallels the guanine nucleotide-binding specificity of the p21.

An important question is whether p21 phosphorylation is an autocatalytic reaction or p21 is simply a substrate for a contaminating protein kinase. Various commonly used protein

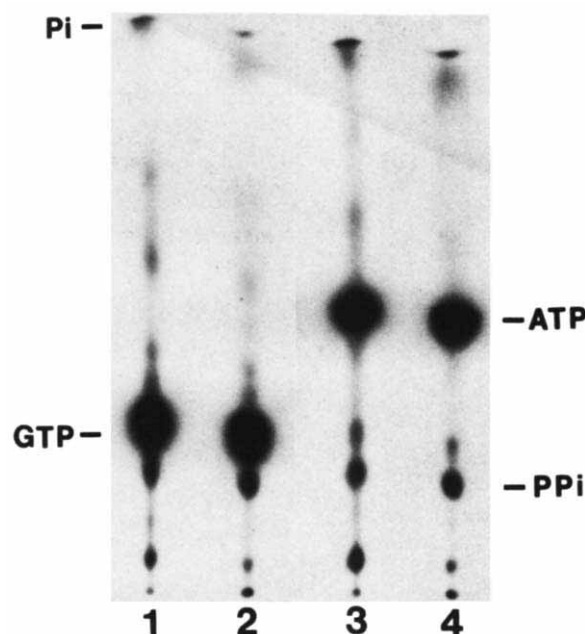


Fig. 3 Analysis of remaining $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ substrates after incubation with the purified p21. $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ ($1.5\text{ }\mu\text{M}$, 37 Ci mmol^{-1} , NEN) or $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ ($2\text{ }\mu\text{M}$, 22 Ci mmol^{-1} , NEN) was incubated at 30°C for 80 min in the presence and absence of p21 purified by phenyl-Sepharose in conditions identical to those described in Fig. 4A legend. At the end of the reaction, $2\text{ }\mu\text{l}$ of each reaction mixture was spotted on a PEI-thin layer plate (Brinkmann). Ascending chromatography was in $0.75\text{ M KH}_2\text{PO}_4$, pH 3.4. Authentic markers of GTP, ATP, P_i and PP_i were run on the same plate. Lane 1, $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ without p21; lane 2, $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ with p21; lane 3, $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ without p21; lane 4, $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ with p21.

kinase substrates were included in the reaction mixtures, for example phosvitin (40K) in Fig. 2a and b and casein ($\sim 25\text{K}$) in Fig. 2c and d. No GTP/dGTP-dependent phosphorylation of these protein kinase substrates was seen at the p21 peak fractions. Also, the autophosphorylation reaction of the p21 *per se* was not affected by the presence of casein or phosvitin. No GTP-specific kinase activity of the p21 fractions was observed with calf thymus histones, protamine or a synthetic poly-L-tryptophan-L-glutamate copolymer (data not shown). Other characteristics of p21 phosphorylation, such as relative insensitivity to EDTA, *N*-ethylmaleimide (NEM) and other agents, distinguish the autocatalytic reaction from most cellular protein kinases. Furthermore, the nucleotide specificity of the phosphorylation reaction is consistent with the nucleotide-binding specificity of p21. Thus it seems that GTP is a substrate for p21 and it is unlikely that p21 phosphorylation is due to a contaminating cellular protein kinase in the purified preparation.

Characterization of the p21 autophosphorylation reaction

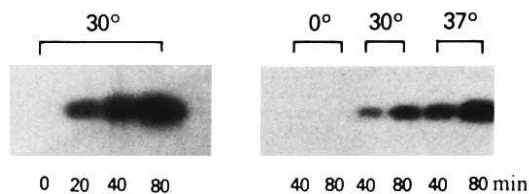
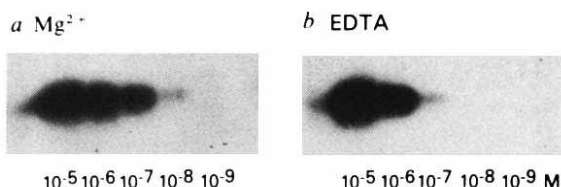
Figure 4A shows the time course and temperature dependence of the autocatalytic reaction. The p21 band becomes more intense with incubation at 30°C for increasing periods of time. The reaction is relatively slow and persistent even after 80 min of incubation. Although nucleotide binding occurs readily at 4°C , autophosphorylation is strongly dependent on incubation temperature. Almost no phosphorylation reaction is seen at 4°C and the phosphorylation reaction rate is higher at 37°C than at 30°C . These characteristics are consistent with a *bona fide* enzymatic reaction. Band intensity was related to the amount of p21 protein, but the exact stoichiometry of phosphorylation is not yet determined. Figure 4B demonstrates the reaction rate as a function of $[\gamma\text{-}^{32}\text{P}]\text{GTP}$ concentration. The p21 bands were cut off from the gels and the radioactivity counted. By double reciprocal plots of the incorporated c.p.m. against GTP concentration, the K_m for Mg^{2+} -GTP was estimated to be $\sim 2 \times 10^{-7}\text{ M}$.

Table 2 Nucleotide binding specificity of p21

^3H -labelled nucleotides	Bound pmol	
	Anti-p21 serum	Normal serum
GTP	2.00 ± 0.09	0.21 ± 0.01
ATP	0.11 ± 0.01	0.09 ± 0.01
UTP	0.09 ± 0.03	0.09 ± 0.00
CTP	0.05 ± 0.01	0.05 ± 0.01
dGTP	2.40 ± 0.09	0.14 ± 0.01
dATP	0.58 ± 0.05	0.06 ± 0.00
dITP	0.08 ± 0.04	0.02 ± 0.00
dCTP	0.05 ± 0.03	0.03 ± 0.00
GDP	2.71 ± 0.33	0.24 ± 0.03
ADP	0.18 ± 0.04	0.16 ± 0.07
UDP	0.23 ± 0.09	0.24 ± 0.11
CDP	0.27 ± 0.13	0.22 ± 0.08
Cyclic GMP	0.05 ± 0.03	0.03 ± 0.00
Cyclic AMP	0.04 ± 0.01	0.03 ± 0.00
Cyclic CMP	0.03 ± 0.01	0.01 ± 0.00

The p21 purified by phenyl-Sepharose column ($10\text{ }\mu\text{g}$) was reacted with $7\text{ }\mu\text{l}$ of antiserum or control normal serum in ^3H -GDP binding assay conditions as described in Table 1 legend. The nucleotide concentration was at $4\text{--}10\text{ }\mu\text{M}$ at a specific radioactivity of $10\text{--}24\text{ Ci mmol}^{-1}$ (NEN). A triplicate determination was performed for each nucleotide and the average values and their standard deviations are listed.

A Time and temperature

B K_m 

C Effect of agents

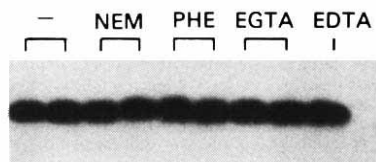


Fig. 4 Characterization of the p21 autophosphorylation reaction. **A**, Time course and temperature dependence of the reaction. The p21 peak fraction (1.5 μ g) from the phenyl-Sepharose column was incubated at 30 °C for 0, 20, 40, or 80 min in the first experiment, and at 0 °C, 30 °C or 37 °C for either 40 or 80 min in the second experiment. The reaction mixture (50 μ l) contained 5 mM $MgCl_2$, 0.01 M sodium phosphate, pH 6.8, 1 mM DTT and 0.5 mg ml⁻¹ casein. The reaction was started by addition of [γ -³²P]GTP (37 Ci mmol⁻¹, NEN) to a final concentration of 1.5 μ M. The reaction was stopped by 10% cold TCA. The precipitated protein was analysed by SDS-PAGE as described in Fig. 2 legend. Autoradiography was for 18 h with Dupont intensifying screen. Similar results were obtained if 0.01 M Tris-HCl, pH 7.5, was used instead of the phosphate buffer. **B**, Effect of [γ -³²P]GTP concentration. The purified p21 (0.8 μ g) was reacted at 37 °C for 60 min with the following concentration of [γ -³²P]GTP (364 Ci mmol⁻¹, ICN): 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸ and 10⁻⁹ M. **a**, Mg^{2+} -containing buffer of 5 mM $MgCl_2$, 0.01 M sodium phosphate, pH 6.8, and 1 mM DTT. **b**, Mg^{2+} -free buffer: the same composition without $MgCl_2$ and with 2.5 mM EDTA. **C**, The effect of various agents. The purified p21 (1.5 μ g) was incubated in 50 μ l of reaction mixture containing 0.01 M sodium phosphate, pH 6.8, 1 mM DTT, 0.5 mg ml⁻¹ phosphatidylcholine and 1.5 mM of [γ -³²P]GTP for 80 min at 37 °C. In addition, various agents were added before incubation: *N*-ethylmaleimide (NEM, Sigma) at 2.5 mM or 5 mM; 1,10-phenanthroline (PHE, Sigma) at 2.5 mM or 5 mM; EGTA (Sigma) at 2.5 mM or 5 mM; EDTA at 5 mM.

and K_m for GTP in the presence of EDTA $\sim 2 \times 10^{-6}$ M. No obvious Mg^{2+} optimum was observed between 0 and 10 mM in the absence of EDTA. The effect of various agents reactive to several protein functional groups is seen in Fig. 4C. The sulphhydryl group reagent, *N*-ethylmaleimide (NEM), at 2.5 and 5 mM did not affect the reaction. At the GTP concentration used here, various chelating agents for metal ions at 2.5 mM or 5 mM also had little effect on the phosphorylation reaction, that is, 1,10-phenanthroline (PHE) which chelates Zn^{2+} and Fe^{2+} ; EGTA which chelates Ca^{2+} ; and EDTA which chelates Mg^{2+} , Mn^{2+} and Co^{2+} . If the enzyme requires these divalent cations at all, these metal ions must already be very tightly bound to the enzyme. These characteristics further distinguish the autocatalytic activity from most known protein kinases which require sulphhydryl group and Mg^{2+} or Mn^{2+} for their activities, or in Ca^{2+} -dependent protein kinases, the Ca^{2+} for their activities²¹. Addition of $CaCl_2$ to the reaction mixture did not appreciably stimulate the p21 activity but is slightly inhibitory above 5 mM. The pH optimum has also been examined between pH 6.0 and

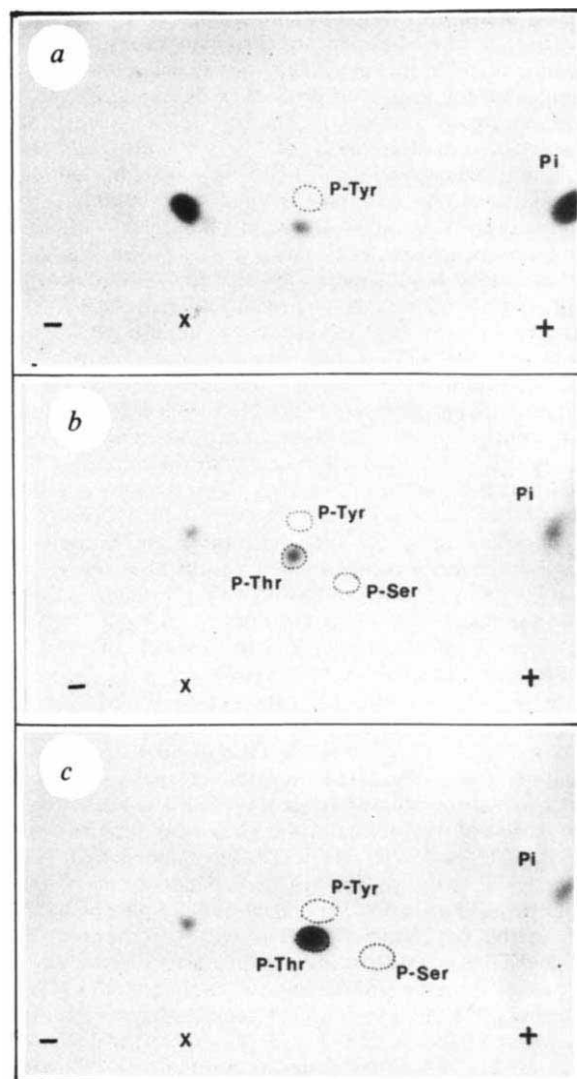


Fig. 5 Analysis of the phosphoamino acids of p21 phosphorylated *in vitro*. The purified p21 from phenyl-Sepharose column was autophosphorylated by incubation with [γ -³²P]GTP as described in Fig. 2a legend. The ³²P-labelled p21 was immunoprecipitated with anti-p21 serum and protein A-Sepharose (Pharmacia). The precipitated protein was solubilized with buffer containing SDS and β -mercaptoethanol. The solubilized protein was further precipitated with 20% cold TCA, washed with ethanol and hydrolysed with 6 M HCl at 100 °C at various time intervals. Approximately 4,000 c.p.m. of lyophilized sample was analysed by the two-dimensional method of Eckhart *et al.*¹⁸ on the thin-layer cellulose plates. $\times$ marks the origin of samples. The first dimension at the horizontal direction was electrophoresis at 950 V for 90 min in pH buffer (glacial acetic acid:formic acid [88%]:H₂O, 78:25:897). The second dimension from bottom to top was chromatography in isobutyric acid:NH₄OH (0.5 M), 5:3. The phosphoamino acid markers, 5 μ g each of phosphothreonine (Sigma), phosphoserine (Sigma) and phosphotyrosine (a gift of Dr Karen Beemon through Dr Gilbert Jay) were mixed with the ³²P-labelled samples and were spotted on the TLC plates. The radioactive spots were detected by autoradiography (6 days) with Dupont intensifying screen and the marker amino acids were visualized by ninhydrin spray. **a**, 4 h hydrolysis in 6 M HCl; **b**, 8 h hydrolysis; **c**, 15 h hydrolysis.

pH 9.5. No sharp maximum was noted and phosphorylation decreased slightly above pH 8.5, perhaps due to alkaline lability of the phosphate linkage (see next section). The enzymation activity was inactivated either by heating at 95 °C for 10 min on proteolytic degradation.

The phosphorylated amino acid residue

The p21 purified by phenyl-Sepharose was phosphorylated *in vitro* by [γ -³²P]GTP and the p21 was immunoprecipitated with the antiserum. The precipitated p21 was solubilized by SDS and

β -mercaptoethanol and was precipitated with 20% trichloroacetic (TCA) acid at 4 °C. The precipitated protein was hydrolysed with 6 M HCl at 100 °C for various periods of time. The only ^{32}P -labelled amino acid was found to co-migrate with the *O*-phosphothreonine internal marker but not with *O*-phosphotyrosine or *O*-phosphoserine (Fig. 5). The spot moving more slowly towards the anode especially noticeable in the 4-h hydrolysate (Fig. 5a) seems to be an incomplete digestion product, as after longer hydrolysis of 8 h (Fig. 5b) and 15 h (Fig. 5c), the intensity of this spot decreases with concomitant increase in the phosphothreonine spot. Similar results were obtained by analysis of the p21 recovered from SDS-polyacrylamide gels or straight TCA precipitation of the reaction mixture. dGTP also appeared to phosphorylate the same amino acid residue as GTP. Consistent with the phosphorylated residue being phosphothreonine but not phosphotyrosine is the alkaline lability of the phosphate linkage. After treatment of the p21 phosphorylated *in vitro* with 0.1 M NaOH at 37 °C for 30 min, the phosphorylated p21 completely disappeared from gel electrophoresis.

In another experiment (data not shown), the p21 was labelled *in vivo* with ^{32}P -orthophosphate. After immunoprecipitation and SDS-gel electrophoresis, the p21 band was eluted and the amino acids analysed. The phosphorylated amino acid residue was threonine, similar to the *in vitro* product. Furthermore, a two-dimensional tryptic peptide map of the *in vitro* phosphorylated p21 was observed to be identical to a two-dimensional tryptic peptide map of the p21 labelled by ^{32}P -orthophosphate *in vivo* (unpublished observation). Several phosphopeptides were observed in each map and the maps were identical. However, as some of the phosphopeptide spots may represent incomplete trypsinization, the exact number of phosphorylation sites on each p21 molecule remains to be determined.

Discussion

The GTP-specific autophosphorylation of p21^{src} observed here seems to be a unique enzymatic activity among those reported for many other transforming proteins. First, the nucleotide substrate specificity of the p21 reaction is more restrictive than the substrate specificity of either the p60^{src} of RSV¹²⁻¹⁵, the p120 of Abelson MuLV¹⁹, or the T antigens of adenovirus¹⁶, polyoma virus^{17,18,22} or SV40 (refs 23-25). The protein phosphorylating activities associated with each of those transforming proteins use as phosphoryl donor ATP and in most cases also GTP, whereas p21 uses only GTP. Second, the protein kinase activity of the partially purified RSV p60^{src} and the SV40 large T antigen has been demonstrated to phosphorylate exogenous protein acceptors such as casein, phosphatidylserine or immunoglobulin heavy chain in soluble systems²³⁻²⁷. No such activity has been detected in reactions containing p21. Third, the phosphorylating enzymatic activities associated with p60^{src}, the Abelson MuLV p120 and the T antigens occur rapidly at 4 °C (refs 15, 19).

Although binding of GDP or GTP to p21 can be observed at 4 °C, phosphorylation is markedly temperature-activated and proceeds linearly for up to 80 min at 37 °C. Finally, the amino acid residue phosphorylated with [γ - ^{32}P]GTP in the p21 is a threonine residue, not a tyrosine residue as observed for the other proteins¹⁸⁻²⁰. Thus at this point, it is not possible to assign a common denominator to the phosphorylating activities associated with transforming proteins. Comparing the results of p21 with other transforming proteins, note that p21 was purified by a GDP-binding assay that does not require the measurement of protein kinase activity. Thus it is possible that our binding assay and/or method of purification have allowed us to discern this novel GTP-specific autophosphorylating activity.

The question, then, is: what is the biochemical activity of p21 that is responsible for cell transformation? Two types of biochemical activities have now been associated with p21: (1) a binding activity in which either GDP or GTP will bind non-covalently to the p21; and (2) an autophosphorylating activity in which p21 is phosphorylated by the gamma phosphate of GTP. It is possible that the nucleotide-binding activity is the first step in the interaction of the p21 with GTP and that the subsequent phosphorylating activity is responsible for its transforming function. Within this model, autophosphorylation could be a phosphoenzyme intermediate which is a step in an enzymatic reaction which normally will go to completion *in vivo* and is trapped in the present *in vitro* condition due to the lack of suitable acceptors or cofactors for the catalytic turnover of the reaction. Alternatively, autophosphorylation of p21 may simply be an autoregulatory mechanism for some other activities of the p21 which may more closely reflect the transforming activity *per se*. The p21 may be activated or inactivated as a consequence of its phosphorylation and p21 activity may function in a way that does not *per se* require phosphorylating activity. Regulatory proteins which bind guanine nucleotides do exist and p21 could function as such a protein in a 'transforming reaction' which itself does not necessarily require phosphorylating activity. The regulatory G-protein of the adenylate cyclase system is an example^{28,29}. Purified p21 and its phosphorylated product reported here may permit one to determine the missing components of its transforming activity. These may be protein substrates, other small or large metabolite molecules which simply bind to the p21-guanine nucleotide complex or actively participate in a unique phosphotransferase or even GTPase reaction. Recently, the Ha-MSV p21 and RSV p60 have been found to be located at the inner surface of the plasma membrane of transformed cells with the p60 being concentrated at gap junctions^{10,30}; the Abelson MuLV p120 has a transmembrane subcellular location³¹ and the polyoma middle T antigen is also membrane-associated³². The generally similar anatomical locations within cells of these transforming proteins suggest that the cell membrane may be a major site of action for transformation. Autophosphorylation of these proteins may have an important role in regulating normal membrane functions.

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Molecular cloning and sequencing of cDNA encoding the precursor to the small subunit of chloroplast ribulose-1,5-bisphosphate carboxylase

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Polyadenylated RNA from leaves of pea (Pisum sativum) has been copied into DNA and cloned in the Escherichia coli plasmid pBR322. From these clones we have identified and sequenced DNA encoding the mRNA for the precursor of the small subunit of the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase.

CHLOROPLAST development requires the coordinated activities of both plastid and nuclear genetic systems. Some chloroplast polypeptides are encoded in the nucleus and synthesized in the cytosol, whereas others are encoded and synthesized in the chloroplast¹. Recent data suggest that chloroplast polypeptides synthesized in the cytosol take the form of higher molecular weight (MW) precursors that are cleaved to the mature form on entry into the chloroplast². One much studied chloroplast protein, which provides a model system for studying nucleus-chloroplast cooperation, is the stromal enzyme ribulose-1,5-

bisphosphate carboxylase (EC 4.1.1.39, also known as Fraction I protein, here abbreviated to RuBPCase). This enzyme is the most abundant chloroplast protein³. In higher plants it occurs as an oligomer of eight large subunits (MW 55,000) and eight small subunits (MW 14,000). The function of the small subunit is unknown, but the large subunit contains the catalytic sites for both the carboxylase and the oxygenase activities⁴.

The large subunit is encoded in the chloroplast DNA^{5,6} and synthesized inside the chloroplast⁷. The small subunit is encoded in nuclear DNA⁸, and synthesized in the cytosol as a precursor of

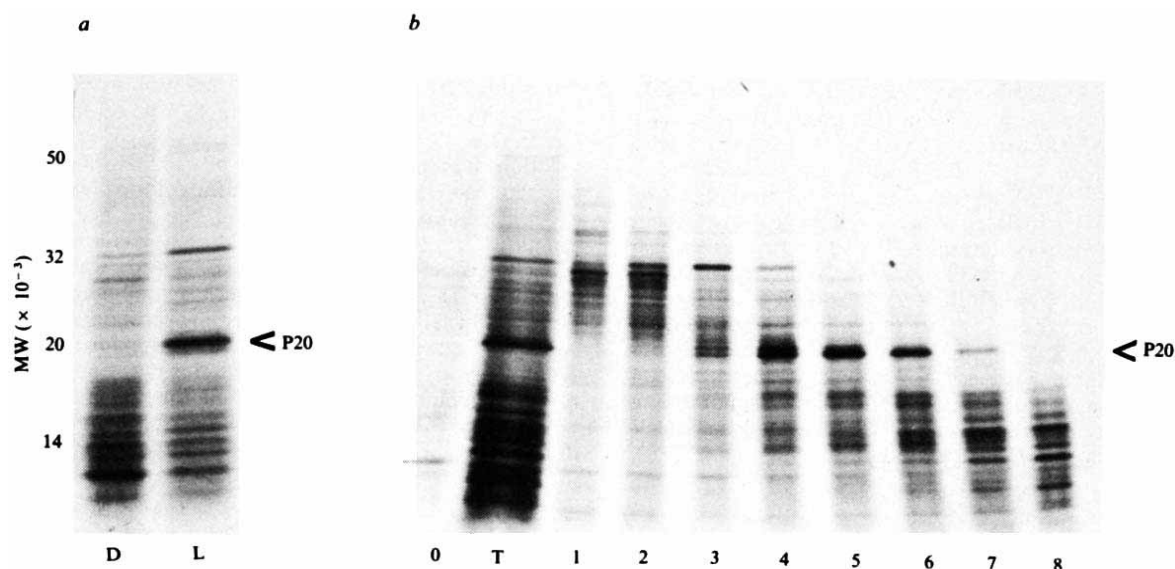


Fig. 1 Translation products of RNA preparations used in cloning and colony hybridizations. *a*, RNA from dark-grown and illuminated pea plants. Polysomal polyadenylated RNA was prepared from shoots of pea (*Pisum sativum*, var. Feltham First), translated in a wheat-germ protein-synthesizing system and the products analysed as described previously^{10,12}. The photograph shows the autoradiograph of an SDS-polyacrylamide gel of the translation products. D, RNA prepared from pea plants grown for 9 days in darkness; L, RNA prepared from pea plants grown for 9 days in darkness followed by 48 h continuous illumination; P20, precursor to the small subunit of RuBPCase. Each sample contains an equal amount of trichloroacetic acid-precipitable radioactivity. *b*, Size-fractionated RNA from illuminated pea plants. Polyadenylated RNA from the leaves of illuminated pea plants (L, above) was fractionated by electrophoresis in a gel (17 × 17 × 0.3 cm) of 1.5% (w/v) agarose containing 0.1 × E buffer²⁶ (3 mM NaH₂PO₄, 3.6 mM Tris, 0.1 mM Na₂EDTA) and 50% (v/v) deionized formamide. Electrophoresis was for 3 h at 30 mA after which the region of the gel containing RNA species between 0.1 and 0.7 × 10⁶ MW was cut into eight equal fractions. Each gel fraction was cut further into 3-mm cubes and placed in a test tube. RNA was eluted from these gel sections (0.2 g each) by shaking at 4 °C with 0.25 ml 10 mM Tris-Cl pH 7.6, 0.4% (w/v) SDS, 1 mM EDTA and 400 mM LiCl. Elution was for a total of 20 h during which time the elution buffer was changed twice. Eluates for each fraction were pooled and the RNA precipitated with ethanol. The RNA was dissolved in 200 mM HEPES-KOH, pH 7.6, and centrifuged for 5 min in an Eppendorf 5412 microcentrifuge to remove any insoluble material. The RNA was ethanol-precipitated from the supernatant and finally reprecipitated once more from this buffer before being dissolved in sterile distilled water for translation and colony hybridizations (see Fig. 2). Translation and product analysis on a SDS-polyacrylamide gel were as described previously^{10,12}. The photograph shows an autoradiograph of the translation products. O, No RNA added; T, unfractionated RNA; 1–8, RNA size fractions of decreasing MW; P20, precursor to the small subunit of RuBPCase.

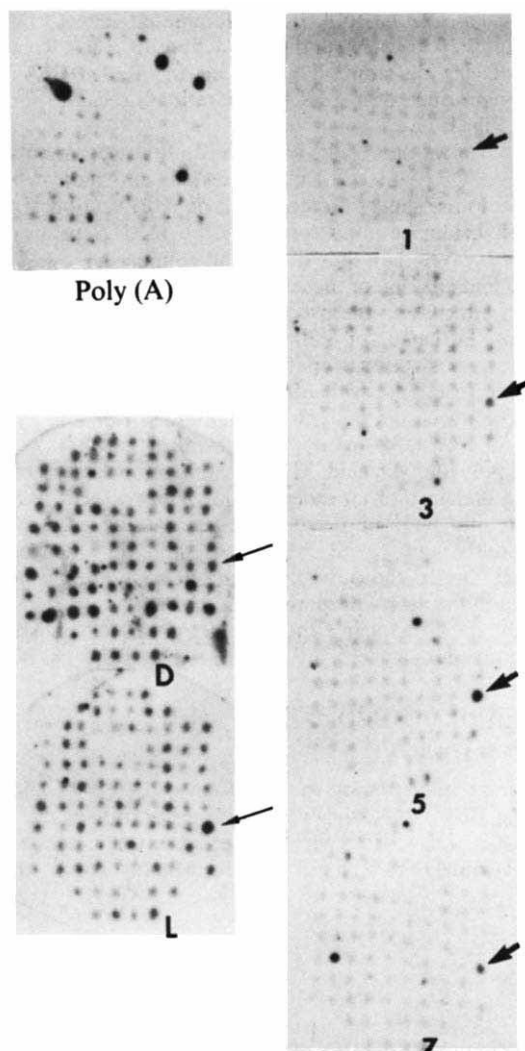


Fig. 2 Isolation and identification of cDNA clones. Polysomal polyadenylated RNA (10 μ g) from illuminated pea leaves (see Fig. 1a) was used to direct the synthesis of cDNA using the method and buffers described by Myers and Spiegelman²⁷. The reaction was carried out in 100 μ l using 40 units of AMV reverse transcriptase. The cDNA was purified by phenol-chloroform extraction, and chromatography on Sephadex G-50 in a buffer containing 0.2 M sodium acetate and 1 mM EDTA. The cDNA fraction was concentrated by ethanol precipitation, dissolved in 70 μ l H₂O, heated to 100 °C for 2 min and cooled in ice water. The denatured cDNA was converted to double-stranded DNA in a 100- μ l reaction mixture containing 50 mM HEPES pH 6.9, 10 mM MgCl₂, 10 mM dithiothreitol (DTT), 0.5 mM deoxynucleotide triphosphates, 200 mM KCl and 10 units of fragment A polymerase I (Boehringer). The reaction mixture was incubated at 37 °C for 120 min, and the DNA purified by phenol-chloroform extraction and chromatography on Sephadex G-50, and concentrated by ethanol precipitation. The double-stranded DNA was treated with S₁ nuclease in a 100- μ l reaction mixture containing 3 M NaCl, 0.3 M sodium acetate pH 4.5, and 1 mM ZnSO₄. The amount of S₁ nuclease required to remove the hairpin loop was determined empirically by titrating against double-stranded cDNA prepared from rabbit globin mRNA, and assaying the length of the product on denaturing polyacrylamide gels. The double-stranded cDNA was incorporated into the vector plasmid pBR322 (ref. 28) in two ways. (1) Using synthetic *Hind*III sequence linkers. Double-stranded cDNA obtained from 10 μ g RNA was dissolved in 10 μ l of a buffer containing 60 mM Tris-HCl pH 7.6, 8 mM MgCl₂, 10 mM 2-mercaptoethanol, 1 mM ATP and 0.2 mM deoxyribonucleotide triphosphates, and incubated with 1 unit of DNA polymerase I for 15 min at 15 °C. 5'-Phosphorylated linkers (60 pmol) in 6 μ l were added to the reaction together with 15 μ l of 60 mM Tris-HCl pH 7.6, 8 mM MgCl₂, and an excess of DNA ligase. The mixture was incubated at 15 °C for an additional 4 h and the nucleic acid purified by phenol-chloroform extraction and chromatography on Sephadex G-200. The cDNA was recovered after ethanol precipitation and ligated to 1 μ g of *Hind*III-linearized pBR322 DNA. The ligated DNA was used to transform the *E. coli* strain HB101 (ref. 29) to ampicillin resistance. (2) By homopolymer tailing of the double-stranded cDNA with dCTP, and *Pst*I-linearized pBR322 DNA with dGTP. The tailing reactions were as described previously³⁰. Double-stranded cDNA from 5 μ g RNA was reacted with 20 units of terminal transferase (PL Biochemicals) in a 100- μ l reaction mixture containing 140 mM potassium cacodylate, 30 mM Tris-HCl pH 7.6, 1 mM DTT, 0.2 mM dGTP or 0.1 mM dCTP, 1 mM CoCl₂, and the plasmid DNA or the cDNA. The reaction was at 37 °C for 30 min. The tailed DNA was recovered by phenol-chloroform extraction and ethanol precipitation, and dissolved in 100 mM NaCl, 1 mM EDTA and 10 mM Tris-HCl, pH 8.0. The pBR322 and cDNA were mixed at approximately equal molar ratios, heated at 60 °C and annealed at 42 °C for 14 h. The annealed samples were used directly for transformation. Bacterial clones containing cDNA chimaeric plasmids produced by the linker method were detected by their sensitivity to tetracycline. Such clones were arrayed on Millipore filters, grown and prepared for nucleic acid hybridization. These filters were hybridized with RNA probes derived from RNA preparations described in Fig. 1 legend. Radioactive RNA probes were prepared using the 5'-phosphorylation procedure³¹. Samples of RNA (0.5 μ g) were hydrolysed in 5 mM Tris-HCl pH 9.5, 10 mM EDTA and 0.1 mM spermidine at 95 °C for 5 min in a 17- μ l reaction volume. The mixture was cooled, made to 50 mM Tris-HCl pH 9.5 and 10 mM MgCl₂, and added to a tube containing 30 μ Ci of dried [γ -³²P]ATP. After mixing, 4 units of T₄ polynucleotide kinase were added and the reaction mixture incubated for 30 min at 37 °C. RNA was purified from the reaction by phenol-chloroform extraction and ethanol precipitation. Before hybridization to probes, each filter was incubated for 6 h in 2 $\times$ SSC, 100 μ g ml⁻¹ *E. coli* tRNA, and 100 μ g ml⁻¹ polyadenylic acid at 60 °C. Hybridization to each 15-cm filter was carried out in 2 ml of 2 $\times$ SSC containing $\sim 4 \times 10^5$ c.p.m. of probe, 100 μ g ml⁻¹ tRNA, and 100 μ g ml⁻¹ polyadenylic acid at 60 °C for 16 h. After the incubation, the filters were washed in 2 $\times$ SSC, 0.1% (w/v) SDS at 60 °C, dried and exposed to X-Omat H X-ray film. The photographs show the results of hybridizing seven different RNA probes to the same 97 clones, the probes being: Poly(A), polyadenylic acid; D and L, polysomal poly(A)-containing RNA from dark-grown and illuminated leaves, respectively (see Fig. 1a); 1, 3, 5 and 7, size-fractionated poly(A)-containing RNA from light-grown leaves (see Fig. 1b). Clone 160 is indicated by an arrow.

MW 4,000–6,000 larger than the mature polypeptide^{9–11}. This precursor (P20) enters isolated chloroplasts with cleavage to its final size by a mechanism which is independent of concomitant translation^{10,11}, and assembles in the stroma with the large subunit molecules into the holoenzyme^{11,12}. The processing activity which cleaves P20 to small subunit is found in the soluble stromal fraction of the chloroplast¹². These features establish a clear difference between the signal hypothesis advanced to explain the transport of secretory polypeptides across microsomal membranes¹³ and the envelope carrier hypothesis proposed to explain the transport of polypeptides into chloroplasts¹⁰.

The mechanism by which the expression of chloroplast and nuclear genomes is coordinated and the polypeptide transported into the chloroplast can be investigated using molecular cloning to elucidate the physical properties of the relevant genes. Specific hybridization probes can be used to obtain information on the structure and function of their RNA and polypeptide products. A cloned fragment of maize chloroplast DNA encoding the large subunit of RuBPCase has been used to show transcriptional control of the expression of this gene in maize leaf mesophyll cells¹⁴. Here we describe the isolation of cDNA clones of the mRNA for the small subunit of RuBPCase from pea (*Pisum sativum*). The cloned DNA has been sequenced to determine the amino acid sequence of most of the precursor polypeptide, and the sequence used to isolate and characterize P20 mRNA.

Light increases the amount of translatable P20 mRNA in polysomes

Light stimulates the accumulation of RuBPCase in pea leaves¹⁰. Polyadenylated RNA was prepared from polysomes isolated from leaves of pea plants grown in darkness for 9 days, and also from pea plants subsequently exposed to 48 h of continuous illumination. Translation of these RNA preparations in a cell-free protein-synthesizing system derived from wheat germ produced the polypeptides shown in Fig. 1a. A comparison of these translation products shows that the RNA from leaves of plants exposed to 48 h of light directs the synthesis of significantly more P20 than that from dark-grown pea plants. If plants are maintained in darkness for a further 48 h instead of

being illuminated, this change does not occur (not shown). Thus, one result of illuminating etiolated pea plants is to cause an increase in the amount of translatable P20 mRNA in polysomes. The identity of P20 as the precursor to the small subunit of RuBPCase has been established previously¹⁰.

Electrophoresis of these two RNA preparations in a polyacrylamide gel containing 7 M urea shows that the RNA from illuminated pea plants contains a ~900-nucleotide species which is not apparent in that from peas grown in the dark. A densitometer scan of a photograph of this gel indicates that this RNA species represents no more than 2% by weight of the total polyadenylated RNA. This RNA species was tentatively identified as the mRNA for P20 due to its co-electrophoresis (not shown) with a size class of polyadenylated RNA enriched with respect to translatable P20 mRNA (Fig. 1b, track 5). Figure 1b shows that RNA, fractionated by size on electrophoresis in an agarose gel containing formamide, is considerably enriched for P20 mRNA. Such enriched RNA fractions proved important in the identification of cDNA clones of P20 mRNA.

Identification of P20 cDNA clones using RNA probes containing different amounts of P20 mRNA

Clones were prepared from polysomal polyadenylated RNA derived from illuminated pea leaves as described in Fig. 2 legend. P20 cDNA clones were identified from the collection of clones by hybridization to RNA probes differentially enriched for P20 mRNA. Clones were arrayed on nitrocellulose filters and hybridized with ³²P-phosphate-labelled RNA probes using the method described by Grunstein and Hogness¹⁵. The probes included RNA from dark-grown leaves (low or devoid of trans-

latable P20 mRNA), RNA from illuminated leaves (high in translatable P20 mRNA), and size fractions of RNA from illuminated leaves differentially enriched for P20 mRNA. The translation products of the RNA preparations used as probes are shown in Fig. 1.

Figure 2 shows the results of hybridization experiments carried out on 97 of the 278 clones tested, and indicates that different colonies show different levels of hybridization. Certain colonies hybridize more strongly to the mRNA probe from illuminated leaves than they do to that from dark-grown leaves (Fig. 2, D and L). One of these colonies (arrowed in Fig. 2) also hybridizes strongly to the probe derived from the size class of RNA most enriched for P20 mRNA (Fig. 2, 1-7). Colonies having the hybridization properties of the colony arrowed in Fig. 2 were therefore tentatively identified as P20 cDNA clones. To observe the probe-dependent differential hybridization shown in Fig. 2, it was necessary to pre-hybridize the filters with an excess of polyadenylic acid. The hybridization was also carried out in the presence of an excess of polyadenylic acid. Without this treatment, we found that clones containing poly(dT-dA) hybridized strongly to all the probes, and this masked the differences due to the differential amounts of coding mRNA sequences in the RNA preparations. Figure 2 [Poly(A)] shows the results of hybridizing the colonies with ³²P-phosphate-labelled polyadenylic acid.

Purification of P20 mRNA with cloned cDNA

To establish the identity of clones tentatively described above as containing P20 cDNA, the translation properties of the RNA hybridized by DNA from such clones was investigated. Supercoiled plasmid DNA was purified from liquid cultures of the

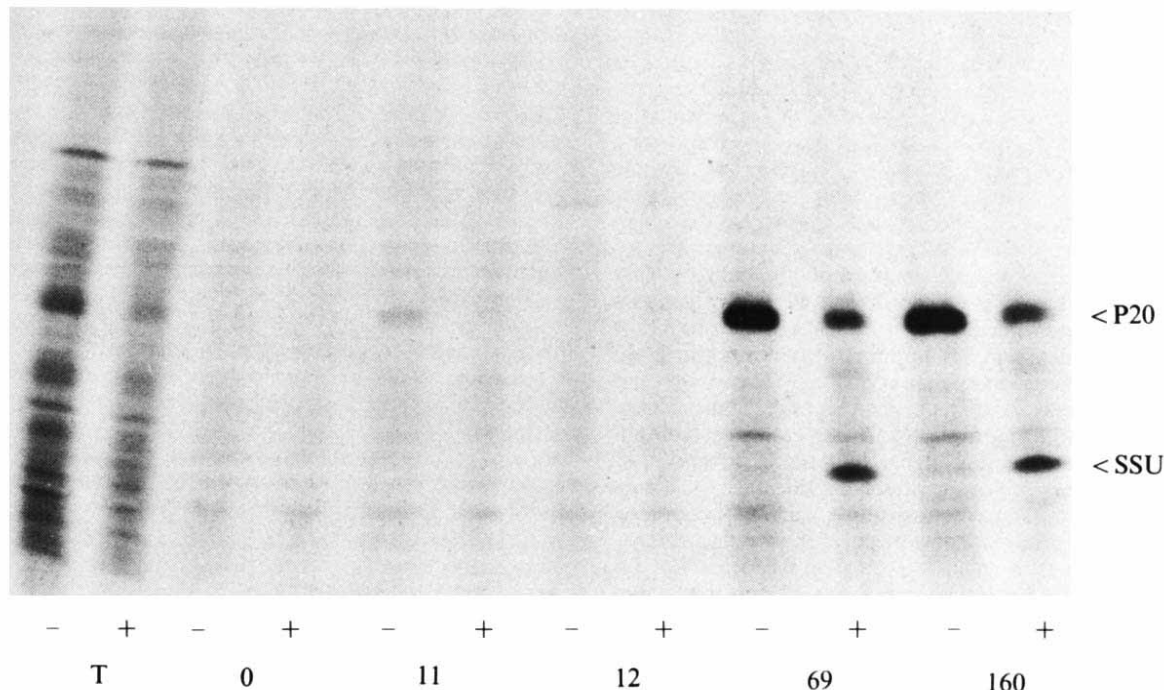


Fig. 3 Identification of clones containing DNA encoding P20. Plasmid DNA was prepared from putative P20 clones by the method of Clewell³². DBM-paper discs were prepared²⁴ and aliquots (20 or 40 µg) of DNA added according to Smith *et al.*¹⁶. A hybrid release translation assay was carried out as described¹⁶, except that hybridization was for 5 h with 50 µg polyadenylated RNA from illuminated pea shoots (see Fig. 1). The purified RNA was collected by ethanol precipitation together with 6 µg *E. coli* tRNA, and finally ethanol-precipitated once from 200 mM HEPES-KOH, pH 7.6, before translation in a 20-µl wheat germ protein-synthesizing reaction mixture^{10,12}. After incubation, the mixture was centrifuged for 10 min at 4°C in an Eppendorf 5412 microcentrifuge and an aliquot of the supernatant solution removed for processing with isolated chloroplasts as described previously¹². Translation products (9 µl) were incubated with 30 µl intact plastid buffer containing washed chloroplasts (6 µg chlorophyll), isolated from 10-day-old pea seedlings¹². The non-processed control consisted of adding 9 µl translation products to an equivalent chloroplast preparation that had been denatured by boiling for 1 min in 1% (w/v) SDS. Products were analysed by SDS-polyacrylamide gel electrophoresis and autoradiography as described previously^{10,12}. The photograph shows an autoradiograph of the products. T, Translation of total polyadenylated RNA; 0, no RNA; 11, 12, 69, 160, RNA complementary to the respective cloned DNA; -, non-processed control; +, post-translational processing with chloroplasts; P20, precursor to the small subunit of RuBPCase; SSU, small subunit of RuBPCase. The DBM-paper disc bearing DNA from clone 11 had only 20 µg DNA added to it, and had been used in two previous experiments. Other DBM-paper discs had 40 µg DNA added and were previously unused.

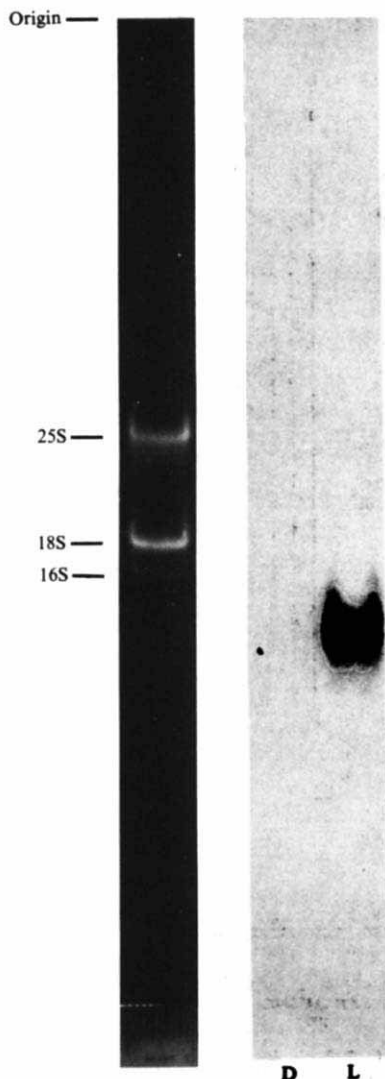


Fig. 5 Identification of P20 mRNA by hybridization with the cloned DNA probe. Samples (2.5 µg) of each of the two RNA preparations whose translation products are shown in Fig. 1a were glyoxalated according to McMaster and Carmichael²³. This RNA was fractionated by agarose-gel electrophoresis, then transferred by blotting to DBM-paper²⁴ for subsequent hybridization to the cloned P20 DNA probe. Plasmid DNA was prepared from clones 60 and 160 (Fig. 4a) as described by Clewell³². DNA was digested with *Hind*III and fractionated by polyacrylamide-gel electrophoresis³³. Gel pieces containing P20 DNA were excised and the DNA eluted at 37 °C for 10 h with 0.5 M (NH₄)₂CH₃COOH, 0.1 mM Na₂EDTA, 5 mM Tris-HCl pH 8.0. The purified DNA was collected by ethanol precipitation, and the different P20 DNA sequences were mixed in equimolar amounts. DNA was radioactively labelled by nick-translation³⁴ using [α -³²P]dCTP (Amersham) to a specific activity of 5×10^7 c.p.m. per µg DNA. Hybridization of this DNA to the RNA immobilized on DBM-paper was as described by Alwine *et al.*²⁴, using 10^6 c.p.m. per 100 cm² paper. After hybridization for 16 h at 42 °C, the paper was washed 3×30 min at 60 °C in $2 \times$ SSC, dried and exposed to X-omat H film (Kodak) with intensifying screens for 2 days. Shown here is a photograph of the autoradiograph. D, RNA from leaves of peas grown in darkness for 9 days; L, RNA from leaves of peas grown in darkness for 9 days followed by 48 h continuous illumination (see Fig. 1a). The electrophoretic mobilities of cytoplasmic ribosomal RNAs (25S and 18S) and 16S chloroplast ribosomal RNA are shown by the UV fluorescence photograph of a track of the same gel in which 2.5 µg total pea leaf RNA was fractionated and stained with ethidium bromide. Low molecular weight ribosomal RNAs are not seen in this RNA because its preparation involved precipitation from 2.5 M NaCl.

contains 44 additional amino acid residues at the N-terminus, but did not investigate the C-terminus. The available DNA sequence to the 5' side of the ATG codon which specifies the N-terminal methionine residue of the mature small subunit encodes 13 amino acid residues. We deduce that these amino acid residues comprise part of a longer N-terminal extension. The elucidation of the complete sequence of this N-terminal extension awaits further molecular cloning.

Polyadenylated mRNAs from eukaryotes commonly contain the hexanucleotide 5' AAUAAA 3', about 20 nucleotides from the poly(A) tail²². Figure 4b shows that no such sequence occurs in the 3' non-coding sequence of P20 mRNA. The 3' non-coding sequence, including the translation termination codon, represents 260 nucleotides. The 3' terminus of pSSU1 contains 98 A residues. Together with the deduced coding sequence of 408 nucleotides, our clones account for 766 residues of P20 mRNA.

Use of cloned DNA as a hybridization probe for P20 mRNA

The strategy for identifying cDNA clones encoding P20 depended on the assumption that the amounts of P20 mRNA in polyadenylated RNA preparations from dark-grown and illuminated pea leaves were reflected in the *in vitro* translation products of those RNAs. The success of this approach implies that our assumption is correct. The availability of cloned cDNA sequences enabled us to check this assumption. Further, we were able to determine the extent to which the size of the P20 mRNA molecule correlates with the cloned sequences

which total 766 nucleotides (Fig. 4a). Equal amounts of the polyadenylated RNAs whose translation products are shown in Fig. 1a were denatured by glyoxalation and fractionated by agarose gel electrophoresis²³. The fractionated RNA was transferred to DBM-paper and hybridized with the cloned P20 cDNA probe labelled by nick-translation²⁴. The results of this analysis are shown in Fig. 5. The probe hybridizes to an RNA species of 900–1,000 nucleotide residues which is observed only in the RNA from illuminated leaf tissue. We conclude that this RNA species is P20 mRNA and that our cloned sequences encode most of this molecule.

The results presented here thus suggest various approaches for increasing our understanding of chloroplast development. We have predicted part of the amino acid sequence of the P20 polypeptide that is removed on entry into the chloroplast^{10–12}. There is no apparent homology between this sequence and that of signal peptide sequences occurring in secreted animal proteins²⁵. This is not surprising in view of the fact that a different mechanism is used to transport proteins into chloroplasts^{2,10}. Furthermore, no significant homology is observed when comparing the pea P20 sequence with that from *Chlamydomonas*²¹. Again, this is not surprising because the *Chlamydomonas* polypeptide is not processed by pea chloroplasts¹¹. Further sequence analysis with other cytoplasmically synthesized chloroplast polypeptides is necessary to determine whether any sequence rules are operating, and how they vary between species. The availability of cloned probes for the small subunit of RuBPCase will allow P20 to be prepared free from other labelled polypeptides, thereby facilitating investigation of its postulated interaction with proteins in the chloroplast envelope¹⁰. The nature of the control of P20 synthesis by light and the structure of the nuclear gene for this chloroplast polypeptide can also now be studied.

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Cell cycle and experimental pattern duplication in the chick wing during embryonic development

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Transient stimulation of cell division within chick wingbud mesenchyme is associated with the reorganization of limb pattern that follows insertion of a small graft from the posterior zone of limb polarizing activity into the anterior border of a host bud. This increase is initiated as an enhanced rate of entry to S phase, correlated with a decrease in packing density among mesenchyme cells, through much of the limb rudiment a few hours after the operations.

GROWTH CONTROL and pattern formation during embryonic development have been largely considered separately in recent years (but see refs 1, 2), because the spatial pattern for differentiation must be determined by direct cellular interactions during early stages, whereas growth has mainly been studied at much later (fetal) stages, where control through circulating hormones of varying degrees of target specificity has been documented^{3,4}. There is, nevertheless, indirect evidence from stages of pattern formation for very local intercellular mechanisms, influencing the rate or amount of cell division in relation to the positional information or position value⁵ that is determining which parts of patterns the cells concerned are to construct^{6–10}.

Relationships between intercellular signal systems controlling the cell cycle and those controlling pattern vary with stage of development. Determination of pattern up to gastrula stages in vertebrates and echinoderms is quite independent of the schedule of cell division which reflects the determination of the pattern rather than determining it^{11–13}. By contrast, in regeneration of insect epidermal patterns after deletion of tissue, replacement and regulation of pattern occur very largely within new tissue specially grown as a response to the disturbance^{6,7}. If generally circulating hormones and their cell-bound receptors mediate growth control in the differentiated and functioning body, while strictly local growth control via cell-bound positional codes is involved in pattern regeneration and the initial building of certain 'second-order' patterns¹⁴ during post-gastrular development, we might expect at certain phases of embryonic life to observe signalling systems for growth which are intermediate between these extremes. We have studied the early wing rudiment in the chick as such a second-order embryonic field^{14,15}, in which pattern is actually being determined during extensive growth.

Within the chick wingbud, grafting a small piece of mesenchyme from a posterior limb-field region (the zone of polarizing activity, ZPA) into an anterior position leads to duplication of the wing pattern about its normal anterior border (Fig. 1)¹⁶. It has been suggested that an increase in the antero-posterior dimension, or width of the outgrowing bud, is a necessary accompaniment of successful duplication¹⁷.

We have examined changes in the cell cycle within the host bud during the very early period after ZPA grafting, and compared this with the effect of a graft from an anterior donor

position similar to the implantation site, an operation causing at most a small pattern disturbance (see later discussion) but usually resulting in quite normal development. The system is a sensitive one for cell cycle studies, as right and left buds are normally closely coordinated in their development¹⁸, and equivalent populations of many thousands of cells can be scanned for uptake after labelling with ³H-thymidine *in ovo*, and scored for mitotic incidence.

Hen's eggs after 3.5 days of incubation (a mixed local strain, Needle Farm, Hamburger & Hamilton, stage 19–20) were windowed and the right limb area of embryos exposed by removal of membranes. Operations grafting a piece of donor right wingbud mesenchyme with overlying ectoderm into a prepared anterior site were performed as described elsewhere¹⁹. In experimental cases the piece came from a posterior region known to possess polarizing activity (opposite somites 19/20, at stage 19), whereas in control operations it was from a donor position similar to the host site, opposite somite 16 at the same stage. Eggs were re-sealed with Sellotape and incubated for 4, 8 or 16 h before onset of ³H-thymidine labelling. Figure 1a shows these operations in diagrammatic form, and Fig. 1b shows the morphogenesis that is finally seen after successful ZPA operations (about 90%) at 10 days of incubation.

The embryos were allowed to incorporate label for 1 h, then fixed. Figure 2 shows the appearances of typical fields within autoradiographs of sections through limb-bud mesenchyme (see legend for details of labelling, fixation, sectioning and staining). Whole buds were dissected free as right-left pairs during dehydration, embedded in Araldite and sectioned transverse to their own proximo-distal axes at 2 µm. All cells visible at each sectional level, distalwards from that where the graft-host junction was visible in the right limb, were scanned as sample populations for labelling index (proportion of nuclei in S phase at some time during the hour before fixation). This was done by dividing the mesenchyme outline concerned into four (or, at the tip, two) parts of equal antero-posterior extent (not equal area) to form boxes. The nuclear population within such boxes was usually between 500 and 1,100, and where fewer cells were transected near the bud tip, pairs of adjacent boxes in the proximo-distal sense were pooled. Data were collected directly with an electronic colony-counter (labelled + total nuclei) and by planimetry, from photographs of standard magnification. The

data boxes are represented in grid plan form for each limb of the pairs shown in Fig. 2. Counts from replica photographs lie within 2% for both cell totals and labelling index, with the latter distributed around 35%. A typical cell packing density is 10,000 cells mm^{-2} of mesenchyme in these transverse sections.

Mitotic index (metaphases per 100 cells) as seen in the toluidine blue images was estimated for the whole population at each sectional level in some limb pairs, from the total cells visible per section (a large number with small relative variation among sections from a level) and the mitoses scored in four to six sections (a small number, of much greater relative variation as it records infrequent events). Estimates of mitotic index lie between 3 and 6% by this method, and are expressed here as % relative difference on the operated in relation to the left side (that is, a mitotic index of 6% is 100% enhancement over 3%). Even with effective samples at each level of 6–10,000 cells, only enhancements of around 25% or more on the right as compared with the left will reach statistical significance ($P < 0.01$), whereas much smaller relative differences in labelling incidence are significant with comparable sample sizes because the absolute incidence is so much higher.

Cell cycle stimulation spreads through host mesenchyme after ZPA grafts

We have analysed in detail two limb pairs labelled between 4 and 5 h after ZPA grafts (5-h limbs), two labelled between 8 and 9 h (9-h limbs) and one labelled between 16 and 17 h (by this stage, selection of visibly successful operations is possible

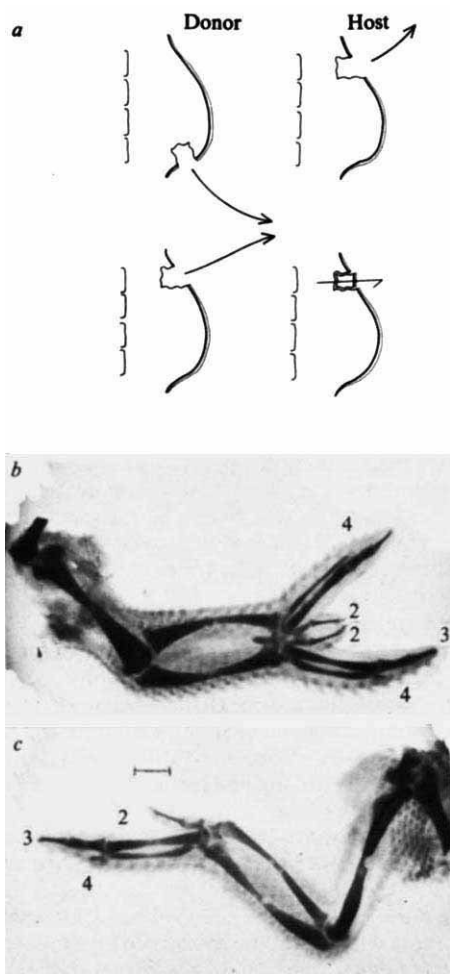


Fig. 1 The grafting operation. *a*, The diagram shows the graft site on the host, and the origin of the graft for ZPA and for sham operations. *b*, Typical result of a ZPA graft at 10 days of development, showing antero-posterior reduplication, together with contralateral control limb. The normal conventions of 2, 3, 4 (relative to a primitive pendent limb pattern of 1–5) are observed for characterizing the digits. Scale bar, 1 mm.

because of the onset of width and shape changes that predict pattern duplication). A further visibly successful 17-h pair has been analysed for mitotic incidence only.

Three anterior → anterior grafted (control) limbs and their left counterparts were examined for S-phase incidence and cell packing density after 8–9 h labelling, and two further 17-h pairs, one control-operated and one after an apparently unsuccessful ZPA graft (in view of lack of shape changes) were analysed for mitotic incidence only. Only host mesenchyme tissue is analysed, the boundary between host and small, discrete graft mesenchymal mass being still evident at these times. The large body of data collected per limb, and the magnitude in biological terms of the effects seen, assures us that our results document real events associated with ZPA implantation.

Figure 3 displays the spatial distribution of labelling index as determined within three of the experimental limb pairs and two of the control-operated pairs. It can be seen that a spatial pattern tends to exist in normal limb buds at these stages (around 21) whereby this index falls off from distal to proximal and from posterior to anterior borders within the mesenchyme. Variation in overall labelling incidence between embryos is unavoidable, probably because of biological variation and variation in precise conditions for ^3H -thymidine uptake during the experimental processing of groups of eggs. There is an enhancement of labelling index in ZPA grafted limbs, relatively local at 5 h, and widespread but concentrated on the anterior side at 9 and 17 h. The effect is not confined to the proximal level of the graft–host junction. A highly comparable sampling of the anatomy is made and 5–10,000 cells are involved in the grand total indices for each limb of each pair, so that the overall enhancements are highly significant ($P < 0.005$), even that within the 5-h pairs. Within individual data boxes, increases in labelling index of 8–10% over the left-hand equivalent (that is, 25–40% relative increase in S-phase entry over the hour, significant at $P < 0.01$) are typical within affected limb regions at each stage. The 9-h pair not illustrated showed an S-phase effect extremely similar on a quantitative and spatial basis to that in Fig. 3, whereas the other 5-h pair showed enhancement near the graft site comparable to that displayed, but could not be analysed spatially due to non-comparable planes of section in right and left buds.

None of the three control-operated 9-h pairs showed an S-phase effect on the right comparable with that seen after ZPA grafting to the anterior site. No overall enhancement was detectable even within the anterior half-mesenchyme total of right as compared with left side (28.4%, 31.6% and 33.9% right anterior half grand total labelling index, compared with 30.2%, 30.3% and 32.0% on left sides, respectively), whereas analysis of each 9-h ZPA pair reveals even posterior half-mesenchymal labelling index to be significantly enhanced within right limbs ($P < 0.01$). We deduce from this that the left is indeed an appropriate control for the right limb within individuals in terms of access of label, which would be essentially via the embryo's circulation. We consistently observe that mean grain density over labelled nuclei appears qualitatively greater in regions (especially in ZPA limbs) of highest labelling incidence among nuclei (see Fig. 2). The rate of DNA synthesis within S phase is probably correlated with the rate of cycle in cells that are susceptible to growth control (see below). The spread of stimulation of entry to S phase, through mesenchymal tissue, is therefore strongly associated with positional disparity between graft origin and implantation site within the rudiment, this disparity being itself generally necessary for the occurrence of new pattern formation as well as the morphological signs of early widening.

Full analysis reveals, however, in one out of the three control-operated cases (Fig. 3), a strong labelling enhancement in certain data boxes adjacent to the graft. This could reflect an effect on the cell cycle of local disturbance to the tissue integrity by the grafting operation, but it may occur because even an 'anterior-to-anterior' graft will induce some cell positional disparity, and any degree of such disparity triggers morphogenetic interactions to some extent (see below).

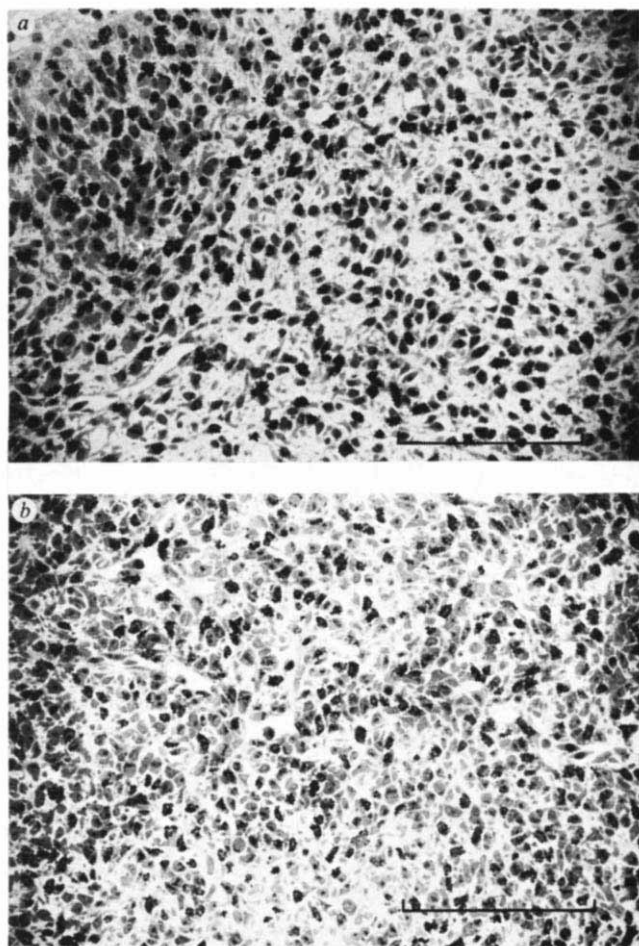


Fig. 2 Typical fields in autoradiographs of a 9-h limb pair. 500 KBq of ^3H -thymidine (TRK 120 [$\text{Me-}^3\text{H}$]thymidine, 720 GBq mmol^{-1}) in 100 μl of warm BSS were dropped into the opened amniotic cavity of each embryo. Eggs were resealed and incubated for 1 h at 38.5 °C. Embryos were then dissected off their chorio-allantoic membranes, removed from the egg and placed in warm BSS for 5 min, during which perfusion of the vascular system with BSS due to continued heart beat and the open circulation acted to lower background radioactivity. They were then fixed in half-strength Karnovsky fluid at pH 7.3, dehydrated in graded alcohols and propylene dioxide and embedded in Araldite. A small set of 2- μm sections from each limb of each pair, parallel to the embryo's long axis, was collected at each 50- or 100- μm interval along the proximo-distal axis, mounted on a subbed slide, dipped in Ilford K2 emulsion, exposed for 14 days, developed in Ilford D19 for 5 min at 20 °C, air dried and stained for 1 min in toluidine blue in 0.1% borax solution at 80 °C. This procedure strongly labels 25–50% of mesenchyme nuclei in various regions of control and experimental limbs, with very low background and no labelled mitoses. *a*, Anterior (pre-axial) region, labelled 8–9 h after ZPA grafting. Labelling within the field is 32% and mean cell density 8,500 per mm^2 . *b*, Anterior (pre-axial) region in the left, control limb for *a*. Labelling index is 23% and mean cell density 11,100 per mm^2 . Scale bars, 100 μm .

Table 1 gives the relative mitotic incidence for the operated as opposed to the left side, in four ZPA grafted pairs and the two 17-h pairs acting as controls. Mitotic enhancement does not become significant before our 17-h time point, but is then dramatic, lying between 50 and 100% at various proximo-distal levels in the two ZPA grafted limbs examined. S-phase entry is responding at least as vigorously in both 9-h limbs (some 25% relative enhancement overall) as at 17 h, and has responded strikingly on a local level by 5 h. These data indicate that the signal caused by newly introduced positional disparity exerts a primary effect on the mesenchymal cell 'cycle' at a point near the passage from a G_1/G_0 condition into the determinate sequence that includes DNA synthesis and culminates in mitosis²⁰. This resembles the situation found for conditions that control growth

of cultured cells, and means that significant extra production of tissue would set in over the period (9–17 h post-operatively) when indeed width change is first observed. None of the control pairs (three 9-h, two 17-h) showed a trace of the massive mitotic effect seen in successful experimental limbs at 17 h.

The greater proportional enhancement of mitotic, as opposed to labelling, incidence seen at the peak of response can be understood by supposing that the actual time spent in mitosis is little changed during faster growth, whereas time taken by S may decrease in partial adaptation to a faster cell cycle^{21,22}. Mitotic incidence might therefore be the more sensitive index of relative cycle time even though ^3H uptake reveals changes closer to the control point in the cycle.

Previous work has documented a general inverse, though complex relationship between mitotic index and cell packing density in normal early wingbuds. Our records of cell packing, in our control and experimental mesenchymes, both confirm this for S-phase and show that where the latter has been stimulated by the morphogenetic interactions we are studying, a correlated decrease of cell packing immediately occurs as exemplified in Fig. 2 (D.S. and J.C., in preparation).

Pattern formation and the control of size in wingbud mesenchyme

Our experiments suggest that, by contrast with a previously studied gastrular embryonic organiser¹¹, the ZPA region may control not only the spatial pattern of fates in limb tissue, but also the production of sufficient tissue to allow establishment of a normal version of pattern.

The postero-anterior decline in mesenchymal S-phase incidence, and the nonlinear aspect of the normal fate-map, whereby tissue whose fate is to form various parts of the structure is 'crowded' towards the posterior in the early bud (see Fig. 4 and refs 23, 24), are both consistent with the idea of a continuous, graded growth signal emanating from the posterior in normal development. However, the growth-stimulating effect of an anterior ZPA graft cannot simply be interpreted as an induction of 'posteriority' in an anterior strip of tissue by

Table 1 Mitotic incidence at each proximo-distal level of right (operated) limb buds, expressed as % relative difference from the homologous left bud level, for five limb pairs

Limb pair	Level	Relative difference
Pair 623 Op. + 5 h	1~	- 17%
	1+	+ 12%
	2	+ 14%
Pair 619 Op. + 9 h	1	+ 26%
	2	+ 18%
	3	- 12%
	4	+ 12%
	5	+ 15%
Pair 634 Op. + 17 h	2	+ 88%*
	3	+ 124%*
	4	+ 62%*
	5	+ 72%*
Pair 240 Op. + 17 h	2	+ 56%*
	3	+ 52%*
	4	+ 49%*
	5	+ 65%*
Pair 632 Op. + 17 h failing to show width and shape change	2	+ 11%
	3	- 17%
	4	- 8%
	5	+ 12%
Pair 633 Sham op. + 17 h	2	- 13%
	3	+ 21%
	4	- 9%
	5	- 15%

Mitoses were pooled from four or six replica sections (toluidine blue, scanned at $\times 250$) and related to the mean number of cells visible at each sectional level. Absolute mitotic index was ~3–6% in this work.

*Only differences between buds in pairs 634 and 240 were significant ($P < 0.01$).

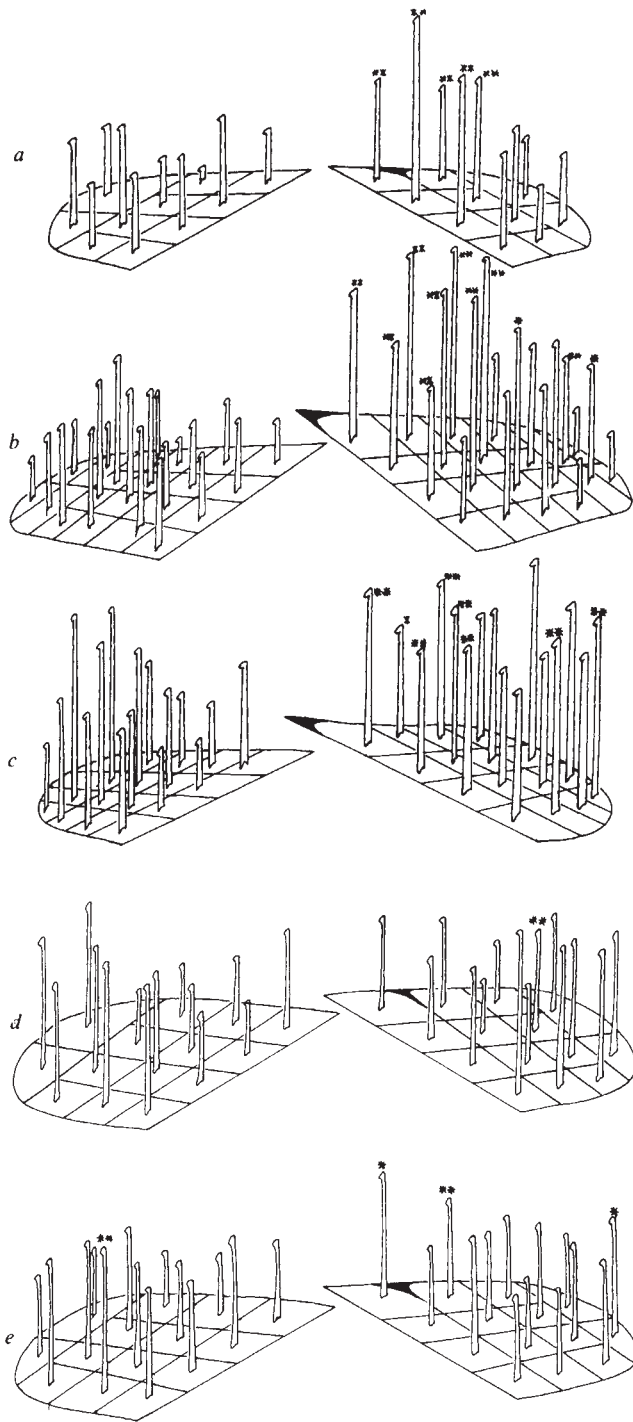


Fig. 3 Spatial variation in labelling index within limb pairs. Each outline represents a single limb-bud with dorsal surface viewed obliquely from the posterior, the (proximal) junction with the body towards the centre. They are arranged in pairs from single embryos, operated ones being on the right-hand side. Each box represents the cell population sampled in an area of mesenchyme derived by dividing the total cross-section visible at that proximo-distal level into four (or sometimes, distally, two) areas of equal antero-posterior width, and thus includes some 500–1,100 (usually around 800) nuclei. Solid corners anteriorly indicate those boxes where graft-host junction was visible. The column height in each box represents the labelling index there, in excess of an arbitrary baseline of 20% (the highest index shown being of 57%, in the 17-h experimental limb). Single and double asterisks above columns signify, respectively, an enhancement over the index of the homologous box in the control limb significant at $P < 0.05$ and $P < 0.01$. Lengths of the proximal baselines of each limb pair are drawn proportional to the total antero-posterior widths of proximal cross-sections, but no attempt is made to depict the detailed shapes of bud outlines. *a*, Limb pair labelled from 4 to 5 h after a ZPA graft; *b*, limb pair labelled from 8 to 9 h after a ZPA graft; *c*, limb pair labelled from 16 to 17 h after a ZPA graft, where preliminary shape changes (indicating width increase) were visible externally in the right bud. *d, e*, Two limb pairs labelled from 8 to 9 h after an anterior → anterior (control) graft.

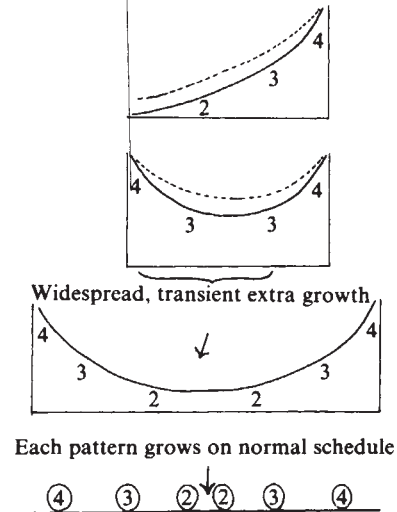


Fig. 4 Outline model for relationship of limb pattern specification to early growth control. Pattern-controlling and growth-promoting signals in the system are seen as distinct entities, and perhaps only loosely correlated in profile at any time, although both organized from the ZPA and of a general gradient form. Cells may respond to the growth signal according to its 'rate of flux' as well as its absolute level, so that cycle stimulation is most pronounced locally soon after ZPA grafts, before the system reaches equilibrium (see, for example, Fig. 3*a*). The process of assigning a complete set of developmental programmes (that is, a whole pattern) to tissue is one that intrinsically occupies a particular space. There may be a stable gradient system of characteristic extent, and also perhaps a pre-pattern of spatial variables^{28,29}, requiring a standard size, for the detailed form. On this view, an anterior ZPA graft might be expected to lead initially to loss of anterior parts, centrally, in a mirror-image duplication. If complete duplication of distal—that is, later-determined³⁰—pattern parts is ultimately found (see Fig. 1*b*), then the antero-posterior extent of territory across which pattern-forming events are occurring must have been appropriately increased by widespread, early response to the extra growth signal. Highly controlled size of the final limb is seen as a result of the constancy of the size at which patterns, for highly programmed growth as well as differentiation, are initially laid down. The first three levels of the diagram represent the normal situation and then two time points within the first, say, 24 h after an anterior ZPA graft. The ordinate is the signal value at each location, the abscissa is the anterior (left) to posterior (right) tissue extent of the limb rudiment at each stage. ---, Profile of a signal influencing the cell cycle. —, Profile of a signal assigning position value. 2, 3 or 4, levels of positional signal associated with formation of particular digits, but cell fate still labile (undetermined). ②, ③ or ④, local tissue determined as, or actually forming, particular digits. The bottom line in the diagram gives the proportions in the later (much larger) developing limb cross-section. Note that growth has been greatest for more posterior elements in the pattern.

pattern re-specification, with an early expression of that altered pattern in terms of intrinsic growth rate. Higher S-phase entry is seen, in regions where we would expect the most rapid changes of position value due to cell interactions at early times, than is found anywhere in the normal limb (for example, 53% labelling index, a 60% relative increase over the left bud as a whole, near a ZPA graft at 5 h after operation). This growth control effect, exaggerated by the unusual positional disparity initially involved in duplication after grafting, largely underlies the early antero-posterior widening whereby both the original and the newly induced limb pattern are able to be complete and to occupy normal amounts of tissue by the time of their specification.

Knowledge of the rate of spread of the growth signal and the kinetics of new tissue production is highly relevant to our understanding of normal and experimental pattern specification in this system. Recent modelling of the antero-posterior pattern control has conceived of the ZPA as source, and limb mesenchyme elsewhere as sink, for a morphogen substance giving a signal gradient of particular profile and spatial extent and hence, by cellular interpretation of local value, a specified differentiation pattern of particular size and proportions^{17,19,24}. Despite recent suggestions that the cell interaction system may also have a component whereby any induced disparity of cell position value (that is, heterotopic grafting) produces some new pattern

formation by local interaction²⁵, there is little doubt about the dominance and primary role of the ZPA in organizing large and potentially complete new patterns when grafted. Although the most pronounced local stimulation occurs very soon after operation (Fig. 3a), the widespread nature of the response thereafter effectively rules out the idea that the new pattern is produced solely in a local zone of special growth, and even makes it unlikely that the entire second pattern is formed within tissue derived from a very small ($\approx 100\text{-}\mu\text{m}$ wide) region taken over by the new organizer. The present results support models of the general gradient form for pattern specification (regardless of molecular nature of the signal) and will help in improving their fit to some of the data.

Figure 4 gives the outline of a model for size control and pattern formation across the limb mesenchyme, which could be tested by future work and which seems most compatible with the data. The ZPA or organizing region is seen as emitting signals, both of essentially graded form but of separate molecular identities, one controlling the fates of cells in the pattern of differentiation, the other the rate and pattern of early growth which may ensure adequate tissue space for the complete specification of pattern by the first signal. The specified, normally sized and proportioned rudiment is then thought to grow according to its own endogenous programme to give the normal anatomy (see above and ref. 9). The ideas are treated further in Fig. 4 legend. Separate, correlated growth and position signals, although not the only tenable hypothesis (D.S., in preparation), would explain (1) the fact that we see widespread, considerable cellular

response in growth within 9 h of grafting, whereas the grafted ZPA must be present for more than 10 h to produce any effect on pattern if excised again, and (2) the avoidance by the system of the loss of anterior pattern values (digit 2), between host and grafted ZPAs, expected on the simplest diffusion gradient models for specification.

Essentially all cells are cycling in the early wingbud²⁶, and the cycle time may be 6–10 h (refs 23, 27). Our data do not allow us to quantify the cycle shortening involved in our experiments but suggest that it is very considerable, and widespread (Fig. 3, Table 1). As total tissue mass only approaches a doubling in the most completely duplicated wings, and each limb pattern is believed to grow to a fixed schedule in late development, the growth stimulation must be very short-lived and followed by a return to equilibrium, perhaps within 24 or 36 h (two or three normal cycle times) of grafting.

We believe this to be the first report of an intercellular growth control system linked to morphogenetic processes in an early vertebrate embryo. Future work should be directed particularly at the question of whether the roles of the ZPA in pattern and in growth control utilize separate, correlated molecular signals, or reflect separate cellular responses to one positional signal system. We are now studying this by more complex experiments in which we attempt to separate the effects of possibly distinct growth signals from those of positional signals.

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LETTERS

Ultraviolet spectra of asteroids

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Spectra over the range 2,100–3,200 Å have been obtained for the three largest asteroids—Ceres, Pallas and Vesta—using the International Ultraviolet Explorer (IUE) satellite¹. These represent the first detailed UV reflectivity data for asteroids. They extend the numerous measurements already carried out in the visible and IR^{2,3} to shorter wavelengths, and so enlarge on previous studies of both the classification and the mineralogy of asteroids⁴.

Table 1 lists basic information concerning the spectra we have analysed. Spectra labelled 'Arizona' were obtained by the University of Arizona: we have acquired them from the World Data Centre {CI} at the Rutherford and Appleton Laboratories.

Those labelled 'UK' were obtained by us at VILSPA, the European ground observatory located near Madrid.

The reduction and calibration of asteroidal spectra has presented a number of problems. In order to obtain the asteroidal contribution, it is first necessary to divide by the solar spectrum. Slight errors in the juxtaposition of wavelength scales in this process can lead to major errors in the residual asteroidal spectrum, and it has proved difficult to achieve exact coincidence. Partly to obviate this, the resolution of the original observations (6–8 Å) has been smoothed in our reduction program to an average resolution of 50 Å. This does not significantly affect our investigation, as solid-body absorption features are much wider than this.

The solar spectrum we have used in our reductions⁵ was obtained in mid-1970, just after a solar maximum. Our spectra were obtained just before maximum. It is possible that this has introduced a systematic error in our results. However, from the data available on variations in the solar UV spectrum as a function of the solar cycle,⁶ we estimate that the colour ratio $\lambda 2,590/\lambda 3,075$ should not differ by more than 5–10% between mid-1970 and the dates of our observations. Variations between spectra of the same asteroid (see Figs 1 and 2) taken at different times can be adequately explained by errors in the observational and reduction processes. There is no evidence that the results are affected by short-term changes in the solar UV flux.

Table 1 Asteroidal spectra analysed

Object	Date observation	Image no.	Exposure time (min)	Aperture	Source
Ceres	28 July 1978	LWR 1890	10	Small	Arizona
Ceres	28 July 1978	LWR 1890	20	Large	Arizona
Ceres	12 November 1979	LWR 6107	10	Large	UK
Pallas	2 June 1978	LWR 1539	16	Small	Arizona
Vesta	21 May 1978	LWR 1532	5	Small	Arizona
Vesta	2 July 1978	LWR 1772	20	Large	UK
Vesta	12 November 1979	LWR 6106	120	Small	UK

The data obtained from IUE were next calibrated to transform them into geometric albedos. This involved four steps. First the radiometric visual albedos from the TRIAD database⁷ were corrected to allow for new estimates of the diameters of Ceres, Pallas and Vesta⁸. Then the visual spectral reflectances of these asteroids (also from the TRIAD database)⁹ were normalized to the absolute visual albedos. After this, the albedos obtained for the three asteroids with the OAO 2 satellite¹⁰, which covered bands in both the visual and the UV, were normalized, over the appropriate wavelength region, to the visual spectral reflectance curves. Finally, our IUE spectra were normalized to the corresponding OAO 2 albedos at 3,075 Å. (One Vesta spectrum {LWR 6106S} was saturated at 3,075 Å and was therefore normalized to the average of the other two Vesta spectra.) These successive steps necessarily introduce uncertainties into the calibration process; as do uncertainties in the IUE calibration, itself¹¹. We estimate that the cumulative random errors from all these sources are $\pm 15\%$. Systematic errors, due especially to uncertainties in the original calibration of the OAO 2 observations, also almost certainly occur, and may be larger than the random errors.

Figure 1 shows our calculated geometric albedo values for Ceres and Pallas over 50-Å intervals. The corresponding values for Vesta are shown in Fig. 2. The estimated error bars are given for one set of measurements only for Ceres and Vesta to avoid confusion in the diagram. The set chosen is in each case the one for which the error estimates are largest. These errors have been derived from measurement of background noise along two strips parallel to, and on either side of, the UV spectrum strip as it appears on the image field recorded by the spectrograph camera. Some data points are missing from the Pallas spectrum, one Vesta spectrum and two Ceres spectra. These correspond to wavelength regions where the original UV spectra are saturated.

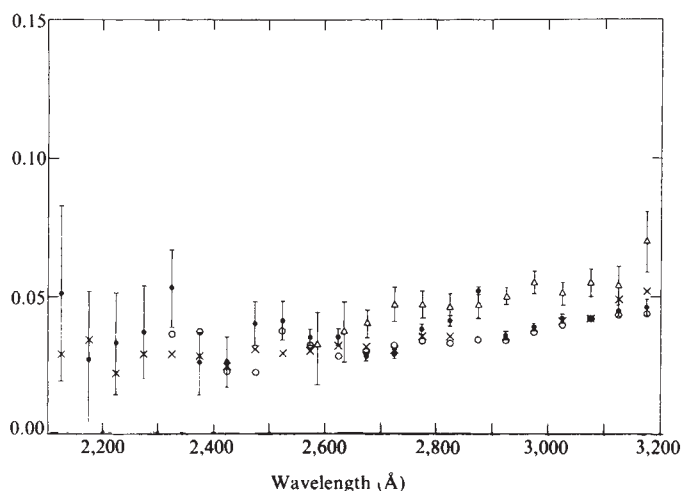


Fig. 1 Values for the geometric albedos of Ceres (●, ○, ×) and Pallas (Δ) over the range 2,100–3,200 Å. ●, LWR 6107 (large slot); ○, LWR 1890 (small slot); ×, LWR 1890 (large slot); Δ, LWR 1539 (small slot).

The results for Ceres presented in Fig. 1 suggest that its reflectivity near 3,200 Å is approximately 0.047. This is some 30% higher than the value obtained from ground-based measurements at 3,300 Å, which not only represents a large and sudden increase, but also goes against the trend of reflectivity in this wavelength region. It seems possible that the OAO 2 albedo at 3,075 Å, which we have used in our calibration, may be in error. D. L. Matson (personal communication) has unpublished data which suggest that the albedo of Ceres at 3,000 Å could be as low as 0.025. Use of this latter value—which corresponds to a lowering of the entire albedo for Ceres in Fig. 1 by a factor of 40%—would resolve the apparent discrepancy in values for the 3,200–3,300 Å region. The shape of the spectral curve for Ceres is not, of course, affected by such a shift: there is some indication of genuine dips centred on 2,425 Å and 2,650 Å.

The OAO 2 calibration for Pallas and Vesta gives results which agree better with ground-based observations. The Pallas spectrum shows no certain features, but there is some indication of a significant change in slope of the Vesta spectrum at about 3,000 Å. Unfortunately, coverage of this region of the Vesta spectrum is poor: further ground-based photometry is needed to define the slope longward of 3,000 Å more accurately.

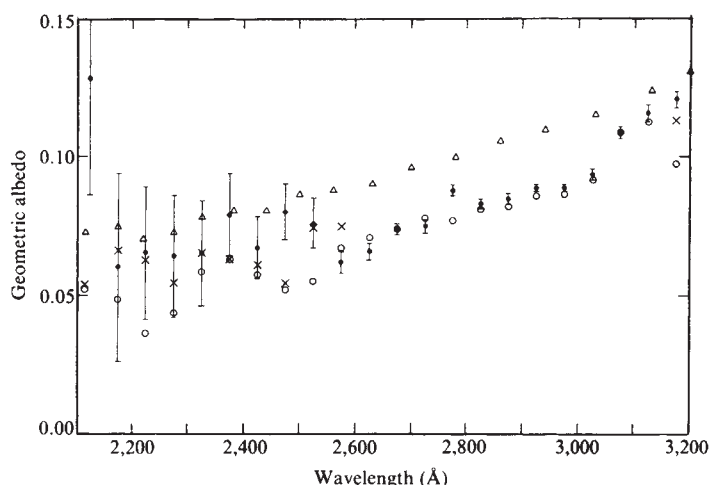


Fig. 2 Values for the geometric albedo of Vesta (●, ○, ×) over the range 2,100–3,200 Å. The spectral reflectance of the meteorite Kapoeta (Δ) over the same range is shown for comparison. ●, LWR 1532 (small slot); ○, LWR 1772 (large slot); ×, LWR 6106 (large slot).

Particular interest attaches to the UV spectrum of Vesta. At visible and IR wavelengths Vesta's spectrum shows considerable similarity to the spectra of the basaltic achondrite group of meteorites⁴. Laboratory measurements of UV reflectivity are available for the meteorite Kapoeta¹², a member of this group, and are plotted in Fig. 2 along with our results for Vesta. The values for Vesta are mainly smaller than those for Kapoeta, though the results for the two bodies suggest a generally similar trend with wavelength.

Systematic differences between the Vesta and Kapoeta spectra may well be attributable either to errors in our calibration procedure, or to differences between the surface texture of the meteorite samples and the asteroid. However, the Kapoeta measurements do not show a change in slope at 3,000 Å, as may occur in the Vesta observations. Confirmation of the existence of this feature might therefore be of importance for the study of the mineral assemblages present on Vesta.

We are currently examining the UV spectra of a number of other asteroids. The main limitation on reduction of this material is that albedo normalization data are only available for the three asteroids discussed above. However, our preliminary work suggests that the UV spectra of asteroids may be as

distinctive as their spectra in the visible and near IR. To the extent that differences in spectral reflectance are due to differences in surface mineralogy, UV observations can therefore be used to extend the knowledge of asteroidal mineralogy already obtained from observations at longer wavelengths.

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Discovery of intrinsic linear polarization in SS433

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The remarkable object¹ SS433 is the optical counterpart of a discrete variable radio and X-ray source^{2–7} located in an extended radio-emitting region (W50) which may be a supernova remnant^{8–11}. The best known attribute of SS433 is the 'moving' emission lines in the visible and near IR spectrum¹². The displacements of the emission features have been shown to be due to Doppler shifts^{12,13} arising from velocity variations with a period of $P \sim 164$ days; velocities up to $+50,000$ (0.17c) and $-35,000$ km s⁻¹ are attained by the redshifted and blueshifted components respectively. A favourable configuration seems to involve two highly collimated, oppositely directed near-relativistic streams emerging from the central object. We report here new observations which reveal that the linear polarization of the optical radiation from SS433 exhibits a long-term variation as well as large night-to-night changes. This establishes that a substantial fraction of the observed polarization is intrinsic to SS433 whereas the remainder may be produced in the interstellar medium. As polarization is an indicator of physical and/or geometrical asymmetries in nature, this phenomenon can have a useful role in discriminating between contending configurations for SS433.

Early attempts to search for polarization of radiation from SS433, mainly as an indicator of magnetic fields and non-thermal processes in general, proved negative. Liebert *et al.*¹³ found no circular polarization in red light, and Thompson *et al.*¹⁴ found no significant linear polarization at 2.2 μ m. Kemp¹⁵ reported $(3.1 \pm 0.6)\%$ linear polarization at $\theta = 3.1^\circ \pm 3.0^\circ$ in the blue-green region of the visible, and Shawl (personal communication) found $(3.96 \pm 0.41)\%$ at $\theta = 4.1^\circ \pm 3.0^\circ$; these values were largely attributed to interstellar polarization. The linear polarization data presented here were obtained with the 2.3-m and 1.55-m telescopes of the University of Arizona

Observatories. These measurements are an order of magnitude more accurate than the above and provide the first clear evidence of variations.

To maximize the signal-to-noise ratio, our observations were made with an unfiltered Ga-As photocathode covering the spectral region between 3,000 and 9,000 Å. As the bulk of the observed optical radiation from SS433 is concentrated at the red end of the spectrum, the characteristic wavelength of our unfiltered passband is near 7,000 Å and thus much redder than those of Kemp and Shawl. This is corroborated by a concordant measurement obtained with a filter having transmission only between 6,500 and 7,500 Å. The exceptional strength of the emission lines may have a small influence on the degree of polarization measured with our unfiltered passband but the effects of varying Doppler shift should be minimal. Also, the polarization at the rest wavelength of H α (6,563 Å) was measured with a narrow passband (32 Å) in two consecutive cycles at times which were coincidentally near phase zero, that is when the emission lines cross the redshift of $z = 0.04$.

Table 1 Optical linear polarization of SS433

Julian Day (2440000+)	(Phase (ϕ))	$p(\%)$	$\theta(\text{deg})$
a, Unfiltered Ga-As, $\Delta\lambda \approx 5,000$ Å			
3,964.99	2.496	2.64 ± 0.20	10.6 ± 2.2
3,966.98	2.508	1.97 ± 0.04	10.6 ± 0.6
4,114.70	3.409	2.18 ± 0.06	11.0 ± 0.7
4,126.64	3.482	1.87 ± 0.04	11.4 ± 0.6
4,127.75	3.489	2.13 ± 0.03	8.5 ± 0.5
4,146.625	3.604	2.03 ± 0.03	7.6 ± 0.5
4,194.585	3.896	2.33 ± 0.04	3.0 ± 0.5
4,217.579	4.036	1.92 ± 0.04	172.3 ± 0.7
4,218.579	4.043	2.15 ± 0.04	174.8 ± 0.5
4,339.986	4.783	2.32 ± 0.02	1.1 ± 0.3
4,349.972	4.844	2.48 ± 0.07	0.7 ± 0.8
4,369.949	4.965	2.41 ± 0.03	178.4 ± 0.4
4,383.944	5.051	2.24 ± 0.02	1.7 ± 0.3
4,384.944	5.057	1.80 ± 0.05	177.5 ± 0.8
4,385.944	5.063	1.47 ± 0.05	174.6 ± 1.0
b, $\lambda = 7,000$ Å, $\Delta\lambda = 1,000$ Å			
4,218.60	4.044	2.22 ± 0.09	176.1 ± 1.2
c, $\lambda = 6,563$ Å, $\Delta\lambda = 32$ Å			
4,217.60	4.037	2.65 ± 0.13	1.6 ± 1.4
4,218.599	4.043	2.61 ± 0.11	1.3 ± 1.2
4,383.944	5.051	2.69 ± 0.11	5.7 ± 1.2
4,384.944	5.057	2.47 ± 0.09	5.7 ± 1.0

The data are presented chronologically in Table 1 where p is the observed percentage of linear polarization and θ is the equatorial position angle. For comparison, ϕ is the 164-day phase¹²; the integer part of the phase is given to show the number of cycles observed. In these data the effects of instrumental polarization are negligible.

Because the observed continuum polarization is the vector sum of at least the intrinsic and the interstellar polarization, it is most instructive to plot the Stokes parameters defined by $Q = p \cos 2\theta$ and $U = p \sin 2\theta$, against time. Any constant interstellar polarization (Q_i , U_i) merely introduces an offset which still allows the intrinsic variations ($Q_*(t)$, $U_*(t)$) to be viewed directly because $Q_o(t) = Q_*(t) + Q_i$ and $U_o(t) = U_*(t) + U_i$. Thus Fig. 1 presents the phase dependence of the observed (continuum) Stokes parameters and shows that large variations in both Stokes parameters do indeed take place over the time scale spanned by the 164-day period but, as yet, the data are still too few to establish periodicity conclusively. The accuracy of our measurements is sufficient to reveal that very short time scale

variations of the polarization are also present. In terms of our current knowledge of SS433 these short-term changes could originate in (1) the underlying binary period (13 days) or (2) stochastic (outburst-like) variations similar to the irregular departures of the radial velocity and light curve¹⁶. Obviously, such short-term variability will have to be averaged-out to enable variations following the 164-day cycle to be observed.

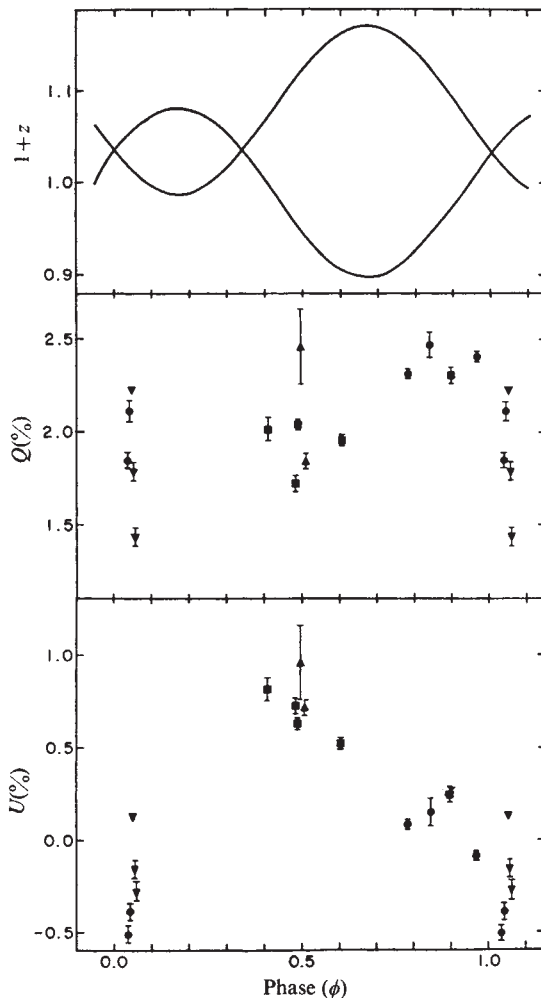


Fig. 1 Wide-band optical polarization of SS433 ($\lambda \sim 7,000 \text{ \AA}$) folded on the 164-day cycle. The variation of the Stokes parameters of the linear polarization $Q = p \cos 2\theta$ and $U = p \sin 2\theta$ are displayed below the radial velocity variations adapted from ref. 12. Observations are in cycles (integer part of ϕ in Table 1): 2, $\blacktriangle$; 3, $\blacksquare$; 4, $\bullet$; 5, $\blacktriangledown$. Large night-to-night variations are particularly noticeable in Q , whereas long-term variability is best seen in U .

In Table 1 the observed degree of polarization at the wavelength of $H\alpha$ is little different from that in the continuum. However, the difference between the position angles of the line and the continuum polarization exceeds the 3σ level. This rotation of the observed polarization at $\lambda 6,563$ can be thought of as an additional term in the expressions for the observed Stokes parameters: $Q_0 = Q_* + Q_{H\alpha} + Q_i$ and $U_0 = U_* + U_{H\alpha} + U_i$. However, without accurate values for the interstellar contributions it is not possible to assess whether the terms $Q_{H\alpha}$, $U_{H\alpha}$ are associated with an enhancement or a dilution of the polarization at $\lambda 6,563$.

From its reddening^{2,17} ($0.75 < E(B - V) < 2.4$), albeit poorly determined, and location near the galactic plane, the allowed range of interstellar polarization (in the mid-visible) for SS433 is

$2.25 > p_i(\%) < 7.20$. An upper limit (3σ) can be estimated using the $2.2\text{-}\mu\text{m}$ IR polarization of $(0.1 \pm 0.1)\%$ observed by Thompson *et al.*¹⁴ at phase $\phi = 0.60$ if one assumes, *a priori*, no source of $2.2\text{-}\mu\text{m}$ polarization in SS433 and that the wavelength of maximum interstellar polarization is close to the normal value, $\lambda_{\text{max}} \approx 0.55 \mu\text{m}$; the result is $p \leq 2.7\%$. (As the optical polarization mechanism is uncertain, a $2.2\text{-}\mu\text{m}$ polarization source in SS433 cannot be dismissed; however, the assumption is consistent with our discussion below.) An examination of polarization survey catalogues¹⁸⁻²⁰ suggests that, in the region of sky containing SS433, the mean position angle of interstellar polarization is $\theta_i \approx 30^\circ \pm 15^\circ$. We have observed the broad band polarization of the uncatalogued stars in the 'diamond' pattern lying only ~ 2 arc min north-west of SS433. These data show that the northernmost component has no polarization, whereas the southernmost and westernmost components are polarized with $p = (1.54 \pm 0.09)\%$ at $35^\circ \pm 2^\circ$ and $p = (1.2 \pm 0.2)\%$ at $16^\circ \pm 5^\circ$ respectively. Thus a lower limit to p_i is $\sim 1.5\%$. Until the interstellar polarization towards SS433 is known with accuracy comparable with the data in Table 1, it will be difficult to solve for the intrinsic polarization in the continuum and at $H\alpha$. However, for values of p_i in the range $1.5\text{--}2.7\%$ at $\theta_i \approx 30^\circ$, the intrinsic polarization at $\lambda 6,563$ (continuum plus $H\alpha$) seems weaker than the continuum and at a position angle at least 15° larger. Because of the same difficulty one cannot yet derive the orientation of the intrinsic plane of polarization for comparison with that of the radio lobes in W50.

Several sources of intrinsic polarization in SS433 have been ruled unlikely. The lack of evidence for strong optical circular polarization ($< 0.1\%$ in broad bands, $< 1\%$ in emission lines) eliminates magnetic fields of $\sim 10^8 \text{ G}$ detected in degenerate dwarf stars¹³. Fields of $\leq 10^4 \text{ G}$, found in Ap-type stars, may still be present²¹ and could be detected with high resolution Zeeman spectropolarimetry. However, polarization in the continuum should be nil. Low degrees of linear polarization due to gyro radiation at radio frequencies are possible²¹ but again with no detectable effects in the optical continuum.

Scattering processes commonly polarize optical radiation in many astrophysical scenarios. Milgrom²² has suggested that light emitted by the regions producing the moving emission and rest-emission lines respectively can scatter from free electrons (Thomson scattering) in any of the other emitting regions and hence become polarized. Margon *et al.*¹², however, have searched for the predicted extra lines and find no conclusive evidence for them.

In the absence of any evidence for dust¹⁴ and in view of the ionization levels expected in the line-emitting regions the most probable source of polarization in the optical continuum of SS433 seems to be electron scattering. Optically thick electron scattering could occur in a dense accretion disk. On the other hand, if the system includes a close binary undergoing mass transfer plus collimated, near-relativistic beams emerging in opposite directions, then optically thin electron scattering may occur in both regions²³, as in Be stars and some X-ray binaries. The continuum polarization would thus display two time scales of variation; one due to the binary period and a longer one due to the precession of the system. Differential changes in Q and U then provide information on the scattering geometry.

In terms of the models currently proposed²⁴⁻²⁶ for SS433, the electron density (N_e) is sufficient to produce significant Thomson scattering. The corresponding optical depth τ needs to be equal to or larger than the fraction of polarized flux, that is $\tau \geq 0.01$ according to the data in Table 1. Now, $\tau = \sigma_T N_e L$, where L is the characteristic size of the scattering region. Then $N_e \geq 1.5 \times 10^{22} \text{ L}^{-1} \text{ cm}^{-3}$. If the scattering region and the source of radiation are close, L is approximately equal to the size of a typical circumstellar shell ($L \sim 10^{12} \text{ cm}$), as is known for Be-shell stars. In this case $N_e \geq 1.5 \times 10^{10} \text{ cm}^{-3}$. However, for scattering blobs relatively distant from the star dilution effects are important and the characteristic length becomes $L \sim l^3/R^2$, where R is the distance to a scattering region of volume l^3 . If such scattering regions can be identified with the emitting

regions of the jets, then the estimated sizes^{24,25} fall in the range $10^{12} \leq l \leq 10^{15}$ cm, with $R \geq 20l$ for good collimation. Therefore, in this second case $10^9 \leq N_e \leq 10^{13}$. Optically thin electron scattering near the emitting regions is also consistent with the energy flow in the jets if this is less than 10^{40} erg s⁻¹.

These results emphasize the fact that polarization plays an important part in the search for a consistent model of SS433.

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Can galaxy warps be used to provide constraints on halo properties?

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The prevalence of flat rotation curves in external galaxies¹ strongly suggests an extensive but as yet unseen halo component. However, as direct observations of the implied matter distribution have proved very elusive, more indirect methods are needed to constrain its properties. At the same time, the explanation of observed warps in galaxies²⁻⁷ has been intensively studied by dynamists, resulting in the appearance of many rival mechanisms (see ref. 8 for a review). For galaxies with companions, the tidal interaction theory is the most attractive, but fails to account for the observed bending of our Galaxy "by a factor approaching 3" (ref. 9). The problem of the rapid kinematical disruption which results if one follows particle orbits in a self-consistent disk is avoided if one accepts the existence of a dominant spherical (halo) component in the region of the warps^{8,10}. Here, we explore the consequence of separating disk and halo mass distributions using a recently developed theory of bending waves². In this theory, the height of bending allows the functional form of the disk density distribution to be determined. With proper normalization this determination allows the halo density to be inferred from the rotation curve. We will discuss the warps in our Galaxy and in galaxies NGC2841 and M33, for which a relatively complete set of reduced data is available.

For many galaxies, the phenomena of warps start at moderate radii where the halo need not be the dominant contribution to the rotation curve. For these instances, Bertin and Mark² have developed an improved version of the earlier Kulsrud, Mark and Caruso¹¹ bending wave analysis and included disk-halo interactions in the manner of Mark¹². This extended analysis is applicable to a wide range of disk-to-halo ratios. In this way, rather than instilling an initial bias towards dominant haloes, the theory may be used to constrain halo properties.

In the Bertin and Mark analysis, the transfer of angular momentum from disk to halo results in a destabilization of bending modes, much like a flag waving in the wind or the two-stream instability in a plasma. The resulting amplification can be large² and may explain the magnitude of the warp in our Galaxy, as well as the existence of warps in isolated spirals^{5,7}. In this theory, the functional form of the warp is mainly dependent on the inertia (total surface density) of the disk $\Sigma(r)$,

$$H \sim \frac{1}{\Sigma(r)r^{1/2}} g(r) \quad (1)$$

where r is the radial coordinate in the disk, H is the maximum vertical displacement at that radius and $g(r)$ is a smooth, weakly varying part of the amplitude. (For the following comparisons with observation, $g(r)$ may be assumed to be constant².)

The warp should be mostly observed in the outer parts of disks where the inertia is low. Frequently, in this region only the gaseous component is observed. Stellar disks are also observed to be warped^{13,14}, which is quite natural in the context of the theory of Bertin and Mark.

The theory as constructed by Bertin and Mark² has strong analogies with the theory of spiral structure¹⁵. As in any linear theory it is simple to calculate a relative amplitude as a function of radius, but the absolute normalization must be obtained directly from observations. However, equation (1) for the functional form of the amplitude provides a simple observational test which is independent of model parameters.

Figure 1a plots surface density against radius in our Galaxy as derived from equation (1) using the data of Henderson⁴. If we temporarily adopt a flat rotation curve with the circular velocity beyond the solar radius (R_0) equal to 250 km s^{-1} , we find the resulting points are well fitted by an exponential with a scale length of 3.4 ± 0.2 ($R_0/10 \text{ kpc}$) kpc. This procedure is superior to determinations of the disk profile from fitting HI velocity profiles, because nothing is initially assumed about the mass distribution as a function of radius. (Of course, in both cases a rotation curve must be assumed or measured so as to derive distances from the observed line-of-sight velocities.) Assuming the same flat rotation curve, Knapp, Gunn and Tremaine¹⁶ derive a scale length of 3.2 ($R_0/10 \text{ kpc}$) kpc from such fitting of HI profiles with an assumed exponential mass distribution. Using the Oort limit¹⁷ to normalize the density at R_0 , we obtain a mass model close to that of Caldwell and Ostriker (in preparation). If we use the most recent rotation curve of Blitz, Fich and Stark¹⁸, the scale length is 4.0 ± 0.2 ($R_0/10 \text{ kpc}$) kpc, the disk mass integrated to 20 kpc is approximately $7.6 \times 10^{10} M_\odot$ (changing by $\sim 10\%$ depending on whether or not a central gap

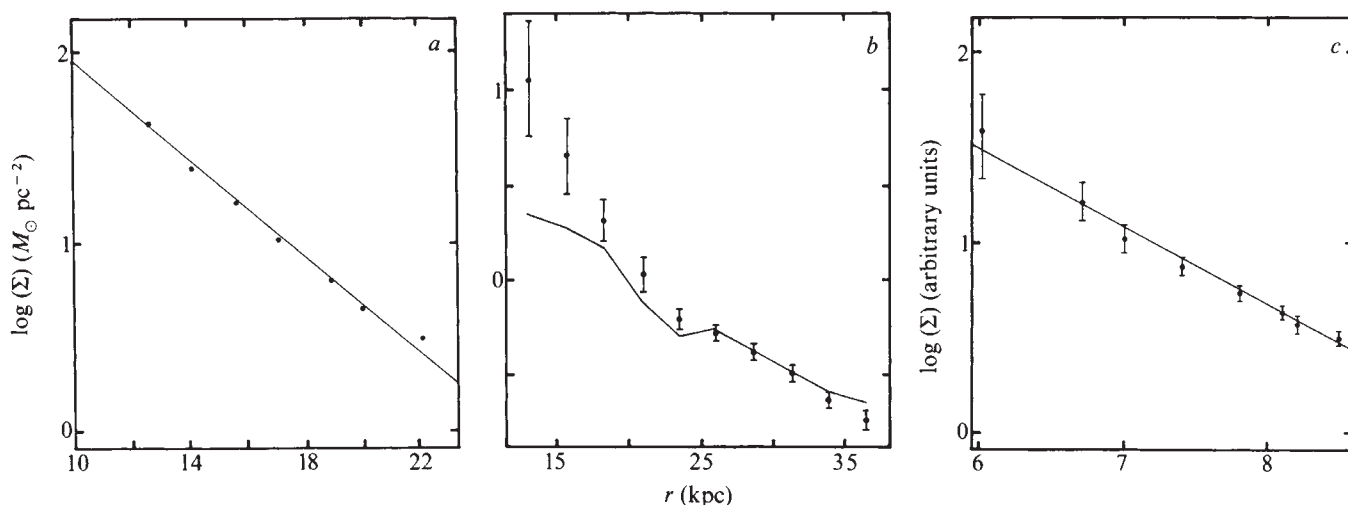


Fig. 1 *a*, The surface density $\Sigma(r)$ against radius in our Galaxy as derived from equation (1) using Henderson's⁴ data for the warp. Distances are determined using a flat rotation curve with the circular velocity at the Sun equal to 250 km s^{-1} . The straight line is an exponential with a scale length of 3.4 kpc, normalized to the Oort limit¹⁷ at the solar radius (10 kpc). Use of the rotation curve of Blitz, Fich and Stark¹⁸ results in a computed scale length of 4.0 kpc. *b*, The surface density against radius for NGC2841 as derived from equation (1) using the data of Bosma⁵ (H_0 is assumed to be $75 \text{ km s}^{-1} \text{ Mpc}^{-1}$). The solid line is the observed neutral hydrogen surface density. The deviations at distances less than 20 kpc may be due to the contribution of the optical disk and (with more accurate data) may provide a method of measuring the mass-to-light ratio of the optical disk. *c*, The surface density against radius for M33 as derived from equation (1) using the data of Rogstad *et al.*⁶. The straight line is an exponential with a scale length of 1.1 kpc.

is included), and the combined mass of the halo and bulge out to 20 kpc is $3.1 \times 10^{11} M_\odot$. If this new rotation curve is used to recompute the halo component in the Caldwell-Ostriker model (leaving their bulge and disk parameters fixed), we find that the fraction of the gravitational force due to the halo at R_0 , $1.5 R_0$ and $2 R_0$ is 17, 42 and 64%, respectively.

Next, we examine the galaxy NGC2841, which has been observed by Bosma⁵. Figure 1*b* compares the surface density of neutral hydrogen with the surface density derived from the observations of the amplitude of the warp, normalized to the *HI* surface density in the outer parts. (All distances in NGC2841 assume $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$.) If there is no systematic variation of the mass-to-light ratio with radius in the observed optical disk and the mass-to-light ratio is less than 6 (twice the values obtained from stellar population models¹⁹), the surface photometry²⁰ suggests that the density of the optical disk is negligible in the region from ~ 20 to 30 kpc; this allows the normalization of the derived density from the warp to the *HI* surface density in the outer parts. However, the optical disk may be the source of the extra mass at radii less than 20 kpc. In any case, the agreement in the range ~ 20 –35 kpc indicates that the *HI* surface density dominates the total surface density in this region. This is only $\sim 1\%$ of the local surface density needed to maintain the observed flat rotation curve⁵, which implies that the mass density of the halo is inversely proportional to the square of the distance, as may be derived from the rotation curve in this region.

Although the bending wave results were derived with the assumption of small inclinations in the warp, it is encouraging that the theory gives a fairly reasonable description of the large inclinations in galaxy M33 (ref. 6). The derived density profile for M33 is shown in Fig. 1*c*. We calculate a scale length of 1.1 ± 0.1 kpc, whereas the photometry yields a value of 1.6 kpc (ref. 21). This discrepancy might be attributed to the high degree of nonlinearity in the bend (the inclination angle of disk changes by 40° in the region from 5 to 9 kpc), but we feel that it is just as likely to be a problem in the photometry. The value of 1.6 kpc quoted by Freeman assumes an angle of inclination with respect to the sky of $i = 55^\circ$, whereas Rogstad *et al.*⁶ found $i = 50^\circ$ and Sandage and Humphreys¹⁴ found $i = 40^\circ$, resulting in scale lengths of 1.4 kpc and 1.2 kpc, respectively. Furthermore, our length scale is derived from the radio data where the warp of the disk has been taken into account⁶ in deriving the disk inclination

as a function of radius. There, effects have not been included in the photometric determination of the length scale optical disk, which is observed to be warped in a manner consistent with the radio data¹⁴. Given these effects, our derived length scale of 1.1 ± 0.1 kpc is quite reasonable.

For galaxies such as NGC2841, it may be possible with better data to measure a mass-to-light ratio in the disks of external galaxies using the bending wave theory. This measurement makes possible a computation of the core radius of the halo component, an important parameter for constraining theories of its origin and composition²². This is possible if the warp is measured over a range where it samples the mass distribution of the optical disk, and extends out to the region where the *HI* surface density dominates and provides the normalization. This project is ideally and uniquely suited for study with the VLA telescope, when accurate 21-cm line observations become possible.

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Identification of the anomalous 26.131-MHz nitrogen line observed towards Cas A

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Konovalenko and Sodin¹ recently reported the detection of an absorption line at a frequency of 26.131 MHz towards Cassiopeia A and attributed the line to the 26.127 MHz $F = 5/2 \rightarrow 3/2$ hyperfine transition of ^{14}N . The difference in frequency corresponds to a radial velocity of -40 km s^{-1} , which is compatible with the mean velocity of the Perseus arm H I absorption lines seen towards Cas A. However, to explain the strength of the line, Konovalenko and Sodin require $(\text{N}/\text{H}) = 8 \times 10^{-3}$ compared with the cosmic ratio of 1.2×10^{-4} . An anomalous abundance of this magnitude would have substantial impact on ideas about galactic nucleosynthesis. We suggest here that the line is a 631α recombination line due to neutral atoms of heavy elements—either carbon atoms in the H I clouds or possibly heavier atoms (Mg, Ca, Fe) in hot ($\sim 10^4 \text{ K}$) ionized gas along the line of sight to Cas A.

The C631 α line lies at 26.126 MHz and the analogous lines of the heavier elements lie at 26.127 MHz. Because of the broadness of the observed line ($\sim 2 \text{ kHz}$), a unique line identification cannot be made on the basis of frequency alone. Radio recombination lines are normally seen in emission and not absorption. We discuss below how absorption can occur for transitions of very large principal quantum number n .

First consider carbon atoms in diffuse clouds. The strength of a recombination line will be:

$$T_L = \tau T_c (T_{\text{ex}}/T_c - 1)$$

where τ is the optical depth, T_{ex} is the excitation temperature, and T_c is the brightness temperature of the background continuum source. The optical depth is given by:

$$\tau = 1 \times 10^7 (f/n) b T_{\text{ex}}^{-1} T_k^{-3/2} \exp(157,800/n^2 T_k) E / \Delta\nu$$

where f is the oscillator strength ($f = 120$ for 631α), E ($\text{cm}^{-6} \text{ pc}$) is the emission measure, $\Delta\nu$ (s^{-1}) is the linewidth, T_k is the electron kinetic temperature and b is the departure coefficient from local thermodynamic equilibrium (LTE). For the levels of interest, b is close to unity.

As carbon is primarily singly ionized in H I clouds, we use

$$E = \int n_e n_{\text{C}^+} dl = \bar{n}_e N_{\text{H}} (C/\text{H}),$$

where we will assume the cosmic abundance value $(C/\text{H}) = 3.7 \times 10^{-4}$. Neutral atomic hydrogen in the direction of Cas A has been studied by Griesen³. Using data of high spatial and velocity resolution he found H I column densities between 3 and $6 \times 10^{21} \text{ cm}^{-2}$. Using these values for the H I column densities rather than the lower resolution data of Shuter and Verschuur⁴, the N/H values required by Konovalenko and Sodin's interpretation range from 5 to 2.6×10^{-2} . Molecules such as H_2CO (ref. 5) and CO (ref. 6) have also been detected in this direction, indicating that there are diffuse clouds along this line of sight. Assuming that all electrons come for carbon we find $n_e = 0.1$ for a cloud of density 300 cm^{-3} . This yields $E = 0.07 \text{ cm}^{-6} \text{ pc}$ for a H I column density of $5 \times 10^{21} \text{ cm}^{-2}$. If the C631 α line is formed in LTE at a temperature of 50 K then $S = 110 E = 8 \text{ s}^{-1}$. This compares well with the value of 6 s^{-1} reported by Konovalenko and Sodin. The following more detailed discussion is necessary to establish the plausibility of an excitation temperature near the LTE value.

At very large n , T_{ex} must approach T_k because the cross-sections for electron collisions which change n become large (proportional to n^6 in the regime of interest). For lower (but still large) n , the galactic radiation field becomes important in determining T_{ex} . At still lower n , these processes dominate less strongly and the excitation temperatures tend to become negative. The available calculations^{7,8} extend only up to $n = 500$, but extrapolation to $n = 631$ suggests that collisions and the galactic background control the excitation temperature provided the electron density exceeds 0.1 cm^{-3} . In this limiting case we have for $n \sim 631$:

$$T_{\text{ex}}^{-1} = (R/T_r + C/T_c)/(R + C)$$

Here, T_r is the brightness temperature of the galactic background, R is the absorption or stimulated emission rate ($R = 500 \text{ s}^{-1}$ for $T_r = 10^4 \text{ K}$), and C is the rate for $n = 631 \rightarrow 632$ collisional excitations ($C = 1,670 \text{ s}^{-1}$ for $n_e = 0.1 \text{ cm}^{-3}$)⁹. For $T_k = 50 \text{ K}$, $T_r = 10^4 \text{ K}$ and $n_e = 0.1 \text{ cm}^{-3}$, the above equation yields $T_{\text{ex}} = 65 \text{ K}$ and the line will be in absorption against the hot ($T_c > 10^4 \text{ K}$) background of Cas A. Using $T_{\text{ex}} = 65 \text{ K}$ and $T_k = 50 \text{ K}$, we predict an integrated line strength of 6 s^{-1} , in agreement with the observed value.

The excitation temperature may be lowered and the line strength increased by the influence of a dielectronic-like recombination process involving the fine-structure states of ionized carbon. This effect has been incorporated into level population calculations for $n \leq 200$ (ref. 10) and shown to produce departure coefficients that can exceed unity for $n \sim 200$. At certain larger values of n , the derivative of the departure coefficients will then be negative, and the excitation temperatures will, therefore, be small and positive.

A remaining question is whether the expected breadth of the C631 α line is compatible with that of the observed line. At typical cloud temperatures, and for $n_e > 0.03 \text{ cm}^{-3}$, broadening by electrons dominates that due to other processes. We utilize the method of Griem¹¹ but use the more recent cross-sections of Gee *et al.*⁹ for the n -changing collisions which are the dominant cause of broadening. The FWHM is:

$$\Delta\nu_e = \frac{1}{2\pi} \sum_{n' \neq n} \langle v \sigma(n \rightarrow n') \rangle n_e \\ \approx 200 \text{ Hz } (n_e/0.03 \text{ cm}^{-3}) (T_k/50 \text{ K})^{3/2}.$$

Therefore, $n_e \leq 0.3 \text{ cm}^{-3}$ is compatible with the observed 2-kHz linebreadth.

The fact that the C631 α line agrees so well in frequency, strength, and linewidth (without assuming any anomalies in abundance, excitation or other conditions) with the observations leads us to believe that it is the most likely identification for the 26.131-MHz line towards Cas A.

The physical processes relevant for the alternate possibility—that the line is due to heavier atoms in hot, ionized gases—have been analysed by Shaver¹² in a different context. At the higher temperatures, dielectronic recombination produces large population enhancements in the high Rydberg levels. The populations reach a peak at $n \sim 200$ and decline towards the LTE values for larger n . Very low, but positive, excitation temperatures then occur for $n \sim 631$ and will produce an absorption line when observed towards a strong ratio source. For $N_{\text{H}} = 10^{21} \text{ cm}^{-2}$, $n_e = 0.1 \text{ cm}^{-3}$ and cosmic abundance, Shaver's calculations indicate that an absorption line near the observed strength towards Cas A would be produced by a blend of Ca, Mg and Fe.

Collisions with electrons also dominate line broadening in this case. From Brocklehurst and Leeman¹³ we take for temperatures near 10^4 K ,

$$\Delta\nu = 30 \text{ Hz } (n_e/0.1 \text{ cm}^{-3}) (10^4 \text{ K}/T_k)^{1/2}$$

hence, $n_e \leq 5 \text{ cm}^{-3}$ is compatible with the observed breadth.

Our suggestion that the line of Konovalenko and Sodin is a recombination line can easily be tested by searching for the 630α or 632α lines.

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Electromagnetic spectral determination of the material composition of penetrable radar targets

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The resonance theory of scattering, recently developed for the diffraction of acoustic and elastic waves¹, is applied here to radar backscattering from penetrable targets in an attempt to solve the inverse scattering problem. This means the extraction of sufficient material-composition information from the electromagnetic scattering amplitudes of the returned echoes, to permit target identification. We illustrate the principle for the case of a conducting spherical target coated with a dielectric layer. The principle of our method is to use the resonances contained in each of the partial waves making up the radar cross-section for material characterization purposes. We demonstrate that in the high frequency region, the spacing between consecutive overtones becomes uniform, and is related to the dielectric constant of the coating. The width of these overtones is proportional to the coating thickness. The resonances, therefore, make up a code that we have deciphered to solve an inverse scattering problem for penetrable radar targets of known shape, but unknown material composition.

The electromagnetic situation described here is analogous to the elastic case we considered earlier² where the material composition of fluid fillers contained within cavities in elastic solids, was determined from the resonances in the elastic echoes returned by the filled cavity, as analysed in the light of the theory of resonance scattering¹.

Figure 1 shows a spherical conducting core covered with a layer of dielectric material of dielectric constant ϵ_1 and thickness δ which is illuminated by an incident plane wave of propagation constant $k_0 = \omega/c_0$. The far-field amplitude of the scattered electric field is given by³

$$\vec{E}_{sc} = E_0 \frac{e^{ik_0 r}}{r} \left[\hat{e}_\theta S_1(\theta) \cos \phi - \hat{e}_\phi S_2(\theta) \sin \phi \right] \quad (1)$$

where

$$S_1(\theta) = -i \sum_{n=1}^{\infty} (-1)^n \frac{2n+1}{n(n+1)} \left(a_n \frac{P_n^1(\cos \theta)}{\sin \theta} - b_n \frac{dP_n^1(\cos \theta)}{d\theta} \right) \quad (2)$$

$$S_2(\theta) = -i \sum_{n=1}^{\infty} (-1)^n \frac{2n+1}{n(n+1)} \left(a_n \frac{dP_n^1(\cos \theta)}{d\theta} - b_n \frac{P_n^1(\cos \theta)}{\sin \theta} \right)$$

and where the 'Mie coefficients' are

$$a_n = -\frac{x j_n(x) - i Z_n(x j_n(x))'}{x h_n^{(1)}(x) - i Z_n(x h_n^{(1)}(x))'} \quad (3)$$

$$b_n = -\frac{x j_n(x) - i Y_n(x j_n(x))'}{x h_n^{(1)}(x) - i Y_n(x h_n^{(1)}(x))'}$$

and $x = k_0 a$, where ω is the circular frequency of incident wave; c_0 the light velocity in the outer medium (Fig. 1); c_1 the light velocity in the dielectric coating; θ, ϕ the spherical angles of the observer; $\hat{e}_\theta, \hat{e}_\phi$ the unit vectors in the θ, ϕ directions; E_0 the amplitude of the incident electric field.

Expressions for the modal impedances Z_n and admittances Y_n are given elsewhere³⁻⁵. The target eigenvibrations correspond to the zeros of the real parts of the denominators of a_n and b_n as follows

$$\frac{1}{iZ_n} = \text{Re} \left\{ \frac{[x h_n^{(1)}(x)]'}{x h_n^{(1)}(x)} \right\} \quad \text{and} \quad iY_n = \text{Re} \left\{ \frac{x h_n^{(1)}(x)}{[x h_n^{(1)}(x)]'} \right\} \quad (4)$$

for the *TE* and *TM* modes, respectively. These characteristic equations can be solved for the real eigenfrequencies $x = x_{nl}^{TE}$ and $x = x_{nl}^{TM}$, respectively. The electromagnetic resonances are illustrated in Fig. 2 for a perfectly conducting sphere with a dielectric coating of relative thickness $\delta_r = \delta/a = 0.1$, and dielectric constant $\epsilon_1 = 6$. The backscattering cross-section³

$$\frac{\sigma}{\pi a^2} = \left| \frac{2}{ka} \sum_{n=1}^{\infty} (-1)^n (n + \frac{1}{2}) (a_n - b_n) \right|^2 \quad (5)$$

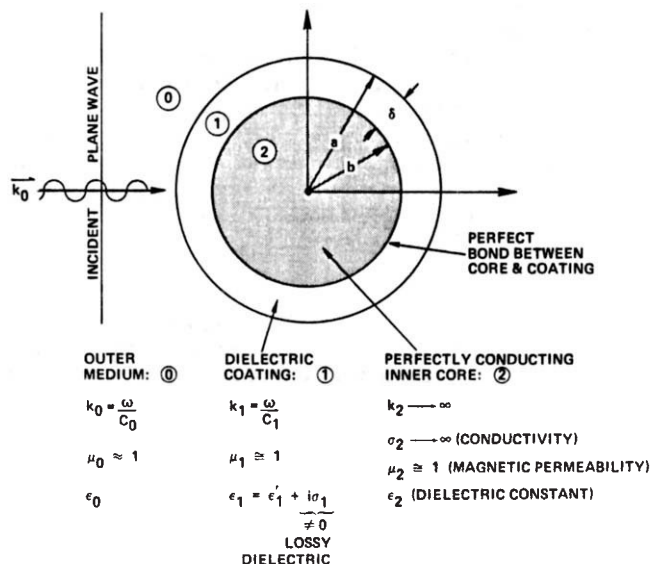


Fig. 1 The geometry of the problem. A perfectly conducting sphere coated with a layer of dielectric material under plane wave incidence.

is plotted in decibels against $k_0 a$ up to 25. We have identified the numerous rapid oscillations visible in Fig. 2 as *TE* or *TM* resonances by actually solving equations (4), and we have marked them by arrows and denoted them by (nEl) or (nMl) labels, respectively. This figure can be viewed as the 'characteristic resonance spectrum' identifying the target. We have also evaluated numerically some of the partial wave coefficients a_n and b_n which exhibit the (nEl) and (nMl) resonances for a given partial wave index n . For $n=10$, Fig. 3 presents the *TE* (top) and *TM* (bottom) moduli $(2n+1)/n(n+1)|a_n|$ and $(2n+1)/n(n+1)|b_n|$ plotted against $k_0 a$, clearly exhibiting some of the resonances. Using the known forms for Z_n and Y_n and the asymptotic forms of the Bessel functions we can now solve the inverse scattering problem. We can show that for $x \gg 1$, one has

$$\frac{1}{iZ_n} \rightarrow (\epsilon_1)^{1/2} \cot k_1 \delta \quad \text{and} \quad iY_n \rightarrow -(\epsilon_1)^{1/2} \tan k_1 \delta \quad (6)$$

where $k_1 = \omega/c_1 = k(\epsilon_1)^{1/2}$. The asymptotic spacing Δ_l between

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adjacent (TE or TM) resonances in this limit can be written as

$$\Delta_l \equiv x_{l+1}^{TE, TM} - x_l^{TE, TM} = \frac{\pi}{(1-\gamma)(\epsilon_1)^{1/2}} \quad (7)$$

where $\gamma = b/a$. A second equation for the two unknowns Δ_l and γ emerges from a consideration of the resonance widths. Our theory¹ gives these widths (for the TE -modes) as follows

$$\frac{1}{2}\Gamma_{nl}^{TE} = \lim_{x \rightarrow x_{nl}^{TE}} \frac{\text{Im} \left\{ \frac{[x h_n^{(1)}(x)]'}{x h_n^{(1)}(x)} \right\}}{\text{Re} \left\{ \frac{[x h_n^{(1)}(x)]'}{x h_n^{(1)}(x)} \right\} - \frac{1}{iZ_n}} (x - x_{nl}^{TE}) \quad (8)$$

and when this expression is evaluated one halfwidth below any resonance (that is, at $x = x_{nl}^{TE} - \frac{1}{2}\Gamma_{nl}^{TE} \equiv X_{nl}^{TE}$) and we use the asymptotic expansion for the Bessel functions ($x \gg 1$) we find

$$(\epsilon_1)^{1/2} = \tan [X_{nl}^{TE}(\epsilon_1)^{1/2}(1-\gamma)] \quad (9)$$

Substituting the value of $(\epsilon_1)^{1/2}$ from equation (7) into equation (9) yields

$$\delta_r = \frac{\delta}{a} = 1 - \gamma = \frac{\pi}{\Delta_l} \cot \left(\pi \frac{X_{nl}^{TE}}{\Delta_l} \right) \quad (10)$$

Substituting this value of $(1-\gamma)$ into equation (7) finally yields

$$(\epsilon_1)^{1/2} = \tan \left(\pi \frac{X_{nl}^{TE}}{\Delta_l} \right) \quad (11)$$

Equations (10) and (11) solve the inverse scattering problem, using our resonance formalism, for the dielectric constant and the thickness of the coating, from the uniform spacings Δ_l and widths X_{nl}^{TE} of the resonances in the high frequency region of the resonance spectrum.

A similar analysis may be carried out for targets of general shape but unknown composition. Shape information may be obtained, as in the case of nuclear photon scattering⁶ from the splitting and relative weights of the resonances. The resonance frequencies of a penetrable prolate spheroidal target, for instance, are split into two different sets $f_{nm}^{(l)}$ and $f_{nm}^{(s)}$, corresponding to standing waves along a long or a short axis,

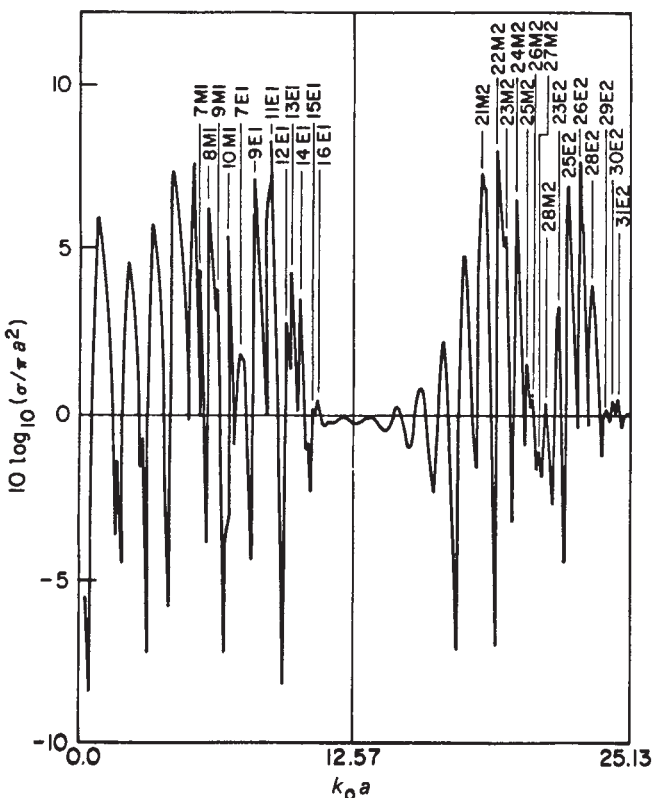


Fig. 2 Radar cross-section (in db) against $k_0 a$ for the coated target in Fig. 1 with $\epsilon_1 = 6$ and $\delta_r = 0.1$. Numerous TE and TM resonances are identified and labelled using our theory¹.

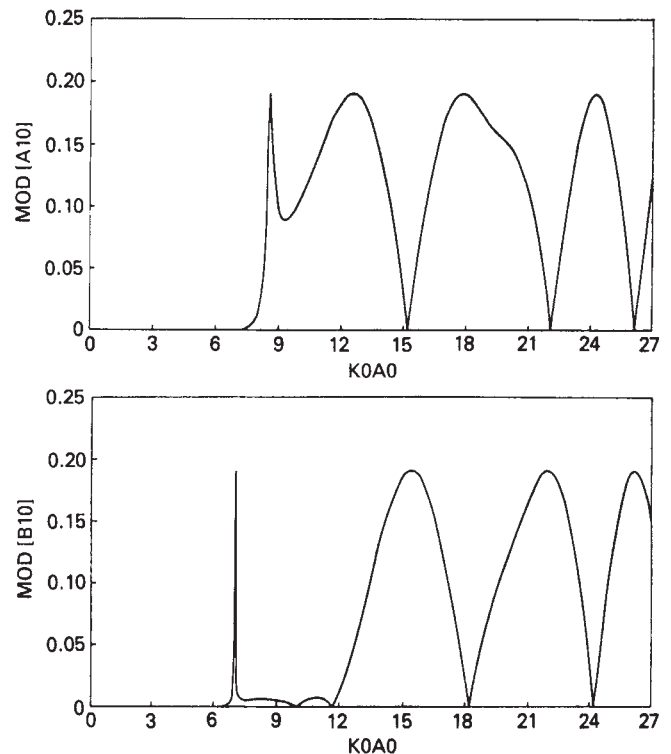


Fig. 3 Typical ($n = 10$) TE (top) and TM (bottom) partial-wave amplitude contained in the cross-section, clearly showing some of the resonances labelled in the (summed) radar cross-section of Fig. 2.

respectively, with $f_{nm}^{(l)} < f_{nm}^{(s)}$. These two sets may be experimentally separated as follows. An incident polarized or unpolarized wave travelling along the long axis will excite only the $f_{nm}^{(s)}$ resonance. A wave incident along a short axis will excite the $f_{nm}^{(l)}$ or $f_{nm}^{(s)}$ resonances, depending on whether it is polarized either along the long or the short axis, respectively; if the wave is unpolarized it will excite both with equal weights. Incidence along any other direction will produce excitation of both sets of resonances with weights depending on the direction of incidence. This allows the lengths of the two axis and the target orientation to be determined. If both the shape and the composition of the target are unknown, these two types of analysis have to be combined.

General areas where our resonance method of target characterization can be applied aside from radar backscattering have been given elsewhere^{1,2}. Examples include the identification of radar targets (such as aircraft types) or of sonar targets (such as types of submerged objects); the identification of mineral or natural gas deposits in seismic prospecting; the characterization of flaws in elastic media by non-destructive evaluation, or of tumours in animal tissue (medical ultrasonics); or in the acoustic design of underwater sound absorbers, among others.

We conclude from the above example that the presence of resonances in the backscattered (radar) cross-section of simple targets can be used to solve material characterization problems in the light of the resonance theory of scattering as recently derived by us¹. This example could be used as the start of a new scientific discipline of electromagnetic spectral analysis for more complex objects.

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Ground-based measurements of atmospheric NO₂ by differential optical absorption

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Nitrogen dioxide is important to stratospheric ozone chemistry¹. The few observations of vertical distribution of the gas are reviewed elsewhere². Most available data relate to ground-based measurements of the total amount in the vertical column. Such observations are ideally made from a high altitude site to avoid local tropospheric sources. Noxon³ observed a mean value of 5×10^{15} molecules cm⁻² from Fritz Peak Observatory. Pommereau and Hauchecorne⁴ obtained much higher values, of a few $\times 10^{17}$ molecules cm⁻², at Haute-Provence Observatory. Noxon *et al.* have reported latitudinal gradients^{5,6} and response to stratospheric warmings⁷, reporting stratospheric column values in the range $1\text{--}5 \times 10^{15}$ molecules cm⁻². During the past year, several series of observations have been made from a low altitude rural site in the UK. Values obtained for the vertical column were in the range $0.8\text{--}5.6 \times 10^{16}$ molecules cm⁻². In view of the tropospheric contribution expected at this site⁸, these values represent an upper limit to the stratospheric NO₂ column and we show here that the values reported by Pommereau and Hauchecorne were anomalously high.

The technique^{3,4,9} used exploits characteristic structure in the absorption cross-section of NO₂ in the spectral range 435–450 nm. Series of direct solar spectra were obtained on clear mornings from sunrise until noon, using a grating monochromator to scan repetitively across this range with 0.5 nm resolution. Partial compensation for changes in atmospheric turbidity during scanning was achieved by normalizing each spectral channel output with respect to a simultaneous broadband solar luminance signal. The ratios were recorded digitally for subsequent computer analysis. A spectrum recorded at high solar elevation near midday, when slant-path NO₂ should be at a minimum, was used as a reference. The difference between the

logarithm of each individual spectrum and the logarithm of this reference spectrum contains the information needed to derive the difference in slant-path NO₂. Care was taken to compensate for wavelength drifts in the monochromator which, because of pronounced solar Fraunhofer structure in the observed spectral range, lead to spurious features in the difference spectra. Difference spectra were corrected for attenuation by atmospheric Rayleigh scattering and by ozone, the latter based on the total ozone amount for the day measured at the same site with a Dobson spectrophotometer. Possible water vapour absorption channels¹⁰ were omitted from the analysis.

Attenuation due to particulate scattering may be assumed to vary linearly with wavelength^{11–13} over the narrow spectral band used. A straight line was fitted to every difference spectrum by the least squares method and the deviation from this line was used as the corrected (difference) spectrum. Corrected spectra were regressed against a spectrum of NO₂ absorption also expressed as a deviation from the best fit line. The shape of this spectrum was determined using the same monochromator and spectral resolution with a carefully purified NO₂ sample, but was quantified by comparison with the absorption coefficients tabulated by Wilkerson *et al.*¹⁴. Regression coefficients thus calculated correspond to the difference in slant-path NO₂ between the times of each solar spectrum and the reference spectrum. A typical series of corrected spectra are shown in Fig. 1, in which successive spectra have had their origin shifted obliquely with respect to the first by an amount proportional to the time difference; the lowest curve is the NO₂ absorption spectrum. The resemblance confirms that NO₂ absorption is being observed. Some of the uncertainty associated with recognizing particular dips and peaks in the attenuated solar spectra^{5,9} is avoided by making full use of the spectrum in this way. Full details of the technique will be published elsewhere.

Differences in slant-path NO₂ relative to the reference, were plotted against the atmospheric mass number, μ , given by the secant of the solar zenith angle at an assumed height for the centre of mass of the NO₂ distribution. Figure 2 is an example of such a 'quasi-Langley' plot, derived from the spectra in Fig. 1. If the NO₂ is constant in time and uniform horizontally, and the assumed height is correct, then the quasi-Langley plot should be a straight line with slope equal to the vertical NO₂ column. Rapid photolysis of NO₂ is believed to occur at sunrise, followed

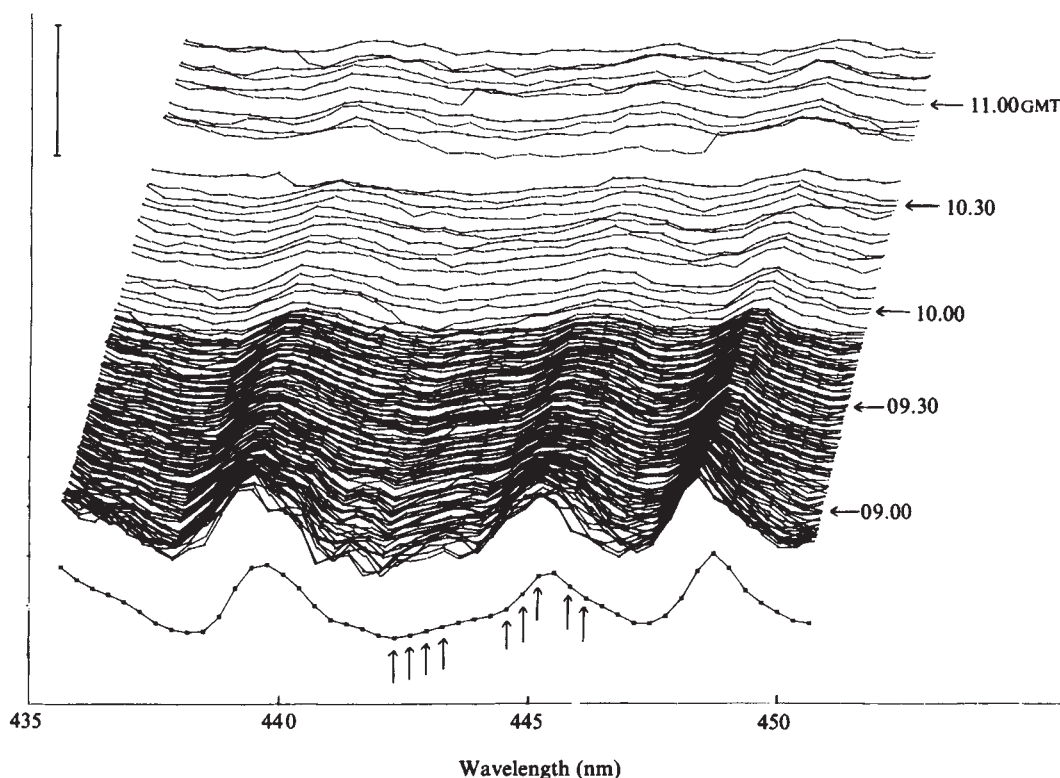


Fig. 1 Corrected spectra obtained using 21 December 1979 solar spectra. The bottom curve represents the NO₂ absorption spectrum used in the regression calculations, after suitable scaling to assist visual comparison with the corrected spectra. Solar spectra span the period 08.52–11.16 GMT on 21 December 1979; sunrise was at 08.06 GMT. Scale bar, optical depth of 0.1, relative to the reference. Note that the amplitudes of differential absorption decrease with time, indicating decreasing slant-path NO₂ during the morning. The smooth appearance of these spectra imply that good wavelength alignment of the solar spectra with the reference spectrum has been achieved. Arrows mark channels omitted from the analysis because of their susceptibility to H₂O absorption.

by a gradual increase in the daytime abundance due to slow N_2O_5 photolysis. Theoretical studies¹⁵ of these processes predict that, by surface sunrise, the effect on slant-path NO_2 due to changes in the vertical NO_2 abundance is small compared with the effect of decreasing slant path, justifying the assumption of constant NO_2 at this time. However, because the above effects are predicted to become comparable near midday, solar spectra obtained within an hour of midday were ignored. Changes in slope were occasionally observed at relatively low μ -values, for which μ is insensitive to the assumed centre-of-mass height. This implies that significant advective changes in the NO_2 column were taking place, probably in the troposphere, which can lead to misinterpretation of the slopes. Consequently only those mornings were accepted on which the slope was essentially linear over a μ -range of at least 3. As our objective was to set an upper limit to the stratospheric NO_2 column, a stratospheric value⁵ of 26 km was assumed for the centre-of-mass height.

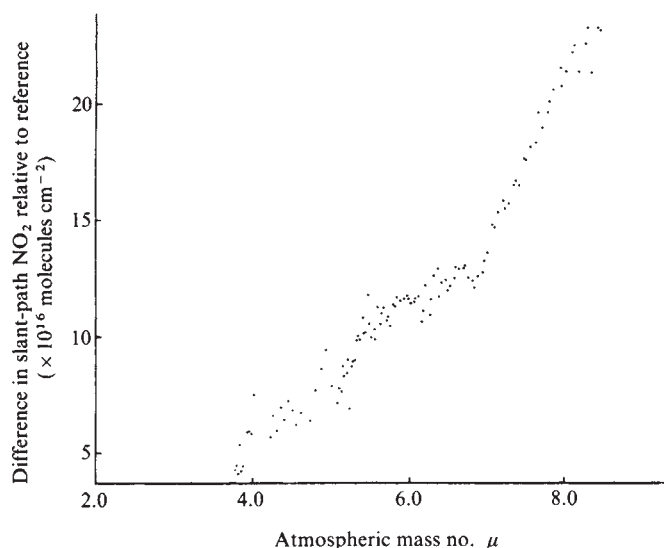


Fig. 2 Quasi-Langley plot for the corrected spectra of Fig. 1. Sunrise corresponds to $\mu = 11.1$. Some of the scatter in the points may be due to horizontal advection of boundary-layer NO_2 but the greater scatter exhibited towards midday (which was often found) may also be due to vertical convection of boundary-layer NO_2 .

During episodes of high boundary-layer NO_2 this height would be much lower, so that our assumed mass numbers will be too low and our tabulated values will be an overestimate of the NO_2 vertical column. This source of bias error, which increases at low solar elevation, has been kept below 20% by ignoring solar spectra obtained at μ greater than 7. Noxon avoids this difficulty by using his twilight zenith sky technique⁵. We have not used this technique as we are not satisfied that in our viewing conditions it will yield valid results.

Estimates of the total NO_2 which were obtained, meeting the above criteria, are presented in Table 1, together with the μ -ranges and corresponding time intervals over which they were evaluated. Values are in the range $0.8\text{--}5.6 \times 10^{16}$ molecules cm^{-2} , which compare with Noxon's^{3,5} findings of a mean total column value of 0.5×10^{16} molecules cm^{-2} and a mean stratospheric column value of around 0.25×10^{16} molecules cm^{-2} . If we subtract this stratospheric value from our totals and assume that the difference is uniformly mixed throughout a surface layer 1 km thick, then the mixing ratios are in the range 2–25 p.p.b. (parts per 10^9). These are within the range of surface mixing ratios (1–34 p.p.b.) reported⁸ at Harwell which is less than 50 km from our measurement site. Noxon's relatively low total column value is understandable in view of the smaller tropospheric NO_2 component expected at his Fritz Peak site (3,000 m a.m.s.l.). However, it is difficult to explain the high values of a few 10^{17} molecules cm^{-2} reported by Pommereau

Table 1 Values of the total vertical NO_2 column at Beaufort Park, Bracknell, Berkshire (51°23' N, 0°47' W, 70 m a.m.s.l.)

Date	Time interval (GMT)	μ -range	Total NO_2 estimated from slope (10^{16} molecules cm^{-2})
6 September 1979	06.10–08.39	1.9–7.0	0.9 ± 0.2
5 November 1979	07.56–08.57	4.0–7.0	0.9 ± 0.3
9 November 1979	08.07–10.44	2.8–6.8	0.9 ± 0.2
21 December 1979	09.11–10.58	3.9–7.0	3.0 ± 0.6
2 January 1980	09.13–10.03	4.0–7.0	1.9 ± 0.8
24 January 1980	09.01–11.12	3.1–6.4	1.7 ± 0.9
4 March 1980	07.29–08.40	3.4–7.0	5.6 ± 0.8
22 March 1980	07.01–08.31	2.6–5.8	0.9 ± 0.1
30 March 1980	07.47–10.00	2.0–5.5	$0.8 + 0.8 - 0.2$
20 April 1980	05.44–06.56	3.0–7.0	1.9 ± 0.4

and Hauchecorne⁴, who observed from Haute-Provence Observatory (651 m a.m.s.l.).

The present apparatus and method of analysis have demonstrated the possibility of measuring the NO_2 total column at a site not having the best of viewing conditions, and have given some support to the relatively low values of NO_2 column reported by Noxon. A spectrophotometer system optimized for this type of measurement is being considered which could be used at a site well removed from continental tropospheric NO_2 sources.

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A modified diabatic circulation model for stratospheric tracer transport

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The possible modifications of the ozone layer by various pollutants has created considerable interest in modelling of stratospheric minor constituents. A major limitation of such modelling work has been the treatment of eddy processes in one- and two-dimensional models. We now describe a two-dimensional model in which some problems associated with the cancellation of the mean and eddy terms in the species continuity equation are removed. If the eulerian mean meridional circulation is replaced by only that part of the circulation driven by the diabatic heating, part of the eddy transport term disappears, although the part associated with the chemistry of the transported species^{1,2} must still be considered. The model explains the results of Prabhakara³ who successfully used a modified diabatic circulation plus Fickian diffusion to model atmospheric ozone.

In the usual eulerian mean approach zonal averages are taken at fixed points in space, defining a latitude–height grid. Thus, with $(\bar{})$ the zonal eulerian mean operator $= 1/2\pi \int_0^{2\pi} () d\lambda$, λ = longitude, and $()'$ perturbation from the zonal mean, any variable χ can be written $\chi = \bar{\chi} + \chi'$. The zonally averaged continuity equation then contains terms involving just the zonal means and other terms (the eddies) involving zonal averages of the perturbation quantities. It has often proved useful to regard the stratospheric circulation as being composed of a zonally symmetric circulation plus eddies, or waves. The two-dimensional eulerian mean continuity equation for a species of mixing ratio χ is written

$$\frac{\partial \bar{\chi}}{\partial t} + \bar{v} \frac{\partial \bar{\chi}}{\partial y} + \bar{\omega} \frac{\partial \bar{\chi}}{\partial p} = -\frac{1}{\cos \phi} \frac{\partial}{\partial y} (\bar{v}' \chi' \cos \phi) - \frac{\partial}{\partial p} (\bar{\omega}' \chi') + \bar{S} \quad (1)$$

where the first two terms on the right-hand side represent the eddy transport of χ . The velocities v and ω are in the y (northward) and p (pressure) coordinate directions respectively, ϕ is the latitude and $\bar{S}$ represents the net sources and sinks of χ .

The treatment of the eddy transport in equation (1) has traditionally followed the procedure of Reed and German⁴ where the fluxes are written in terms of the mean gradients

$$\begin{aligned} \overline{v' \chi'} &= -K_{yy} \frac{\partial \bar{\chi}}{\partial y} - K_{yp} \frac{\partial \bar{\chi}}{\partial p} \\ \overline{\omega' \chi'} &= -K_{py} \frac{\partial \bar{\chi}}{\partial y} - K_{pp} \frac{\partial \bar{\chi}}{\partial p} \end{aligned} \quad (2)$$

with $K_{yp} = K_{py}$. Equation (2) is then used for all quasi-conservative tracers with the same set of K values, generally derived from atmospheric statistics on rather shaky assumptions.

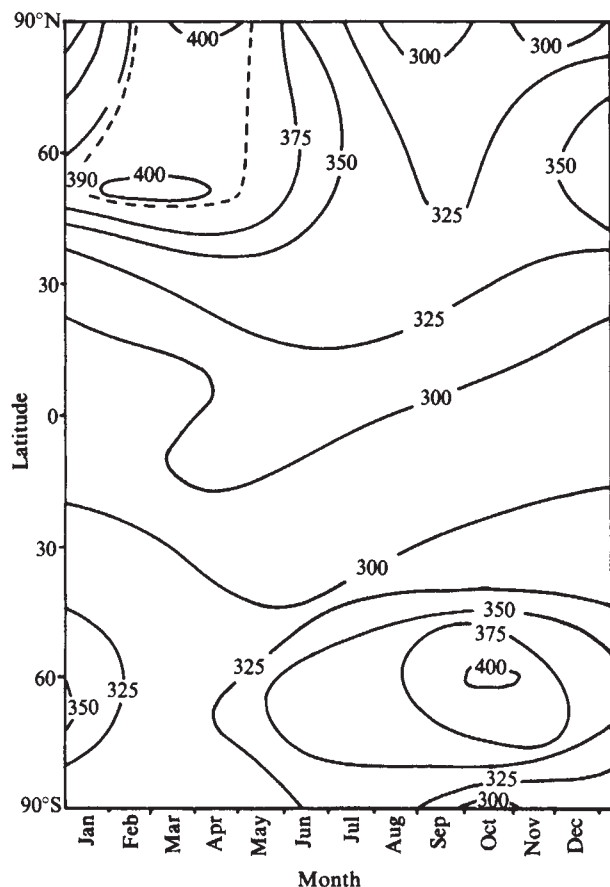


Fig. 1 Latitude–time section of total ozone (m atm-cm) from experiment 1.

Luther⁵, for example, has determined Northern Hemisphere monthly mean values of K from heat flux data, and they have been widely used in modelling studies.

Recent work has suggested improvements to two-dimensional models by using a more physically based K -theory^{1,2,6-8}. By coupling a calculation of planetary wave propagation with solutions of the species perturbation continuity equation. We² have calculated tracer eddy fluxes and hence computed K fields. Of particular interest is that K varies between species, depending on their photochemical lifetimes. Matsuno¹ separated the K values into

$$\begin{aligned} &\text{an anti-symmetric} \begin{pmatrix} 0 & K'_{yp} \\ K'_{py} & 0 \end{pmatrix} \\ &\text{and a symmetric} \begin{pmatrix} K''_{yy} & K''_{yp} \\ K''_{py} & K''_{pp} \end{pmatrix} \text{ component.} \end{aligned}$$

Note that $K'_{yp} = -K'_{py}$ but that $K''_{yp} = K''_{py}$. When the photochemical source is relatively weak, as, for example, with ozone in the lower stratosphere, the anti-symmetric component is independent of the species being transported, depending just on the wave structure: it is applicable to steady, conservative waves and an inert tracer. For photochemical tracers there is the additional symmetric component which depends on the photochemical lifetime, as well as the wave structure, and is, therefore, different for different species. Note that the symmetric component is large and can play a significant role. Matsuno¹ estimated that the anti-symmetric component dominated, but this was due to his choice of 100 days as a species lifetime.

A new approach to studies of tracer transport using a lagrangian framework has been suggested^{9,10} in which the eddy terms apparently need not be calculated. Considering equation (1), in practice the mean and eddy flux terms almost cancel so that the small residual of two large terms must be found. The reasons for the cancellation are well understood in general theoretical terms^{1,6-13}. For steady, non-dissipating waves (the eddies), an eulerian mean meridional circulation is induced which just cancels the effects of the eddies on the mean state. If, however, we consider a lagrangian description, the species continuity equation becomes particularly simple and no eddy terms appear:

$$\frac{\partial \bar{\chi}^L}{\partial t} + \bar{v}^L \frac{\partial \bar{\chi}^L}{\partial y} + \bar{\omega}^L \frac{\partial \bar{\chi}^L}{\partial p} = \bar{S}^L$$

where $(\bar{})^L$ is some lagrangian average. (In a simple case this could be an average following an air parcel. Thus in a lagrangian frame we move with the flow; in a eulerian frame we observe the flow from a fixed point.) The problem now becomes one of finding $\bar{v}^L$ and $\bar{\omega}^L$ (and $\bar{S}^L$) rather than the eddies. Dunkerton⁹ has identified the lagrangian mean circulation with that driven by the eulerian mean diabatic heating. Using the same approximations we can identify a 'residual meridional circulation' (the difference between the eulerian circulation and that part induced by the steady, non-dissipating waves)¹³ as that circulation driven by the diabatic heating.

The cancellation of the eddy terms in equation (1) by an induced mean circulation is only exact in the case of steady waves, with no dissipation, away from critical lines and for an inert tracer. In these circumstances a mean circulation will be induced which will just cancel the transport modelled by the anti-symmetric K components¹⁴. There is, however, no cancellation of the chemically induced transport, modelled by the symmetric K values. The symmetric K component can be shown to be equivalent to the Stokes correction to the eulerian mean source, $\bar{S}$, where $\bar{S}^L = \bar{S} + \text{Stokes correction}$. Thus in both the eulerian and lagrangian description this chemically induced transport must be considered. This is to be expected as they are simply different descriptions of the same physical process.

The three present model experiments all used the same ozone photochemical scheme but included different descriptions of the dynamical processes. All the experiments were carried out

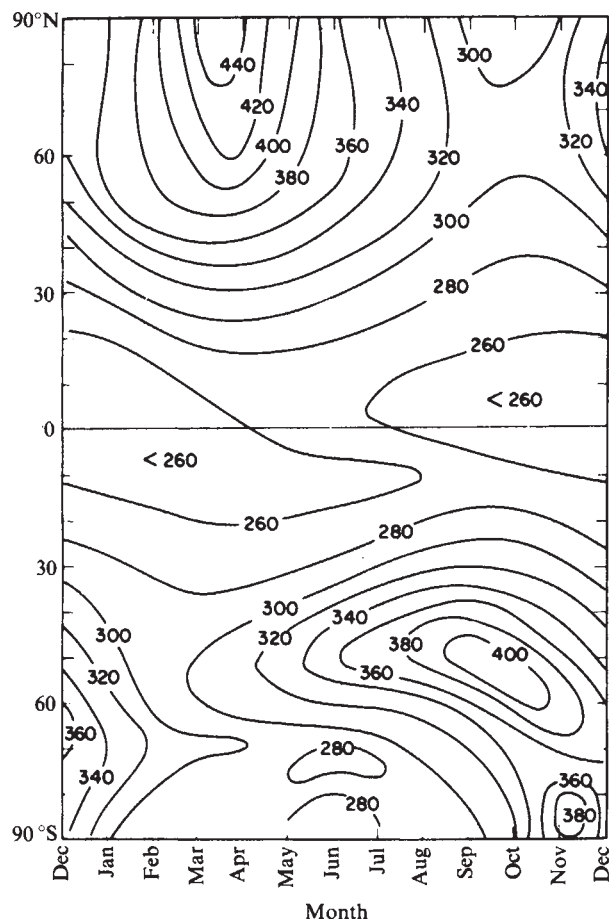


Fig. 2 Observed latitude-time section of total ozone (m atm-cm) after ref. 20.

within the framework of the University of Oxford two-dimensional model¹⁵. The photochemical scheme¹⁶ included the effects of nitrogen and hydrogen compounds.

Figure 1 is a latitude-time section of total ozone from a run in which precomputed values of $\bar{v}$ and $\bar{\omega}$ were used, with the eddy transport calculated using Luther's⁵ values of K . All the species use the same K fields and we assume that $K_{vp} = K_{pv}$. Compared with the observed distribution in Fig. 2, Fig. 1 contains several satisfactory features with an equatorial minimum of total ozone and maxima at the spring pole in the Northern Hemisphere and at $\sim 60^\circ$ S in the Southern Hemisphere about seven months later. This asymmetry is reproduced using observed values of the eddy flux of angular momentum in calculating the $\bar{v}$ and $\bar{\omega}$ fields¹⁷. Although the broad features of the ozone distribution have been reproduced there are, however, important differences of detail. For example, the equatorial minimum should reach lower values whereas the Northern Hemisphere maximum should possibly be higher. This experiment is a typical example of present eulerian mean two-dimensional models.

In the second experiment the eddies were excluded, in both the calculation of the mean circulation and in the species continuity equations. Thus the calculated circulation is driven just by the radiative heating and the release of latent heat. The radiative heating scheme is described in Haigh and Pyle¹⁸. In the lower stratosphere, however, Rodgers¹⁹ heating rates are used. The experiment can be considered from two viewpoints. The diabatic circulation can be considered as the residual circulation after the cancellation of the eddies and the ensuing induced circulation. Alternatively, Dunkerton suggested that the mass transport in the stratosphere is accomplished by the lagrangian mean circulation which he identified to a leading order with the eulerian diabatic circulation, which is used here.

Figure 3 shows the latitude-time section of total ozone from the run. The behaviour is mostly poor. Ozone is moved from equatorial to mid-latitudes where the modelled values are too large. The high latitude values, however, are too low, due to lack of eddy transport. Because negative mixing ratios can develop without any diffusion, the experiment was not run to a steady state. However, in a similar study including diffusion, Prabhakara³ also failed to reproduce the polar maximum with low values of the diffusion coefficients. Maximum values occurred in middle latitudes. Failure to reproduce the observed ozone behaviour in this experiment is evidently not due to the omission of sub-grid scale diffusion.

The omission of eddies in the previous experiment is justified by the cancellation of the eddy circulation and the induced mean circulation. This cancellation does not occur if the waves are growing or decaying, near critical lines or when the tracer being transported is not inert. In a third experiment the uncanceled chemical eddy transport has been modelled. Thus, along with the diabatic circulation, the (chemical) symmetric part of the K -tensor is included. In lagrangian terms, this is equivalent to including the Stokes correction to the eulerian mean source to give the lagrangian mean source. Though we can calculate the symmetric K components², for simplicity we have used Luther's values, which are, of course, symmetric. Our calculated K values² are roughly similar in magnitude to Luther's values in the winter hemisphere, but much smaller in summer.

The total ozone distribution in this run is shown in Fig. 4. The behaviour is good with both an improved equatorial minimum and Northern Hemisphere polar maximum over those found in the first experiment (Fig. 1). By comparison with Fig. 3, the chemical eddy transport plays a major part in transporting ozone polewards.

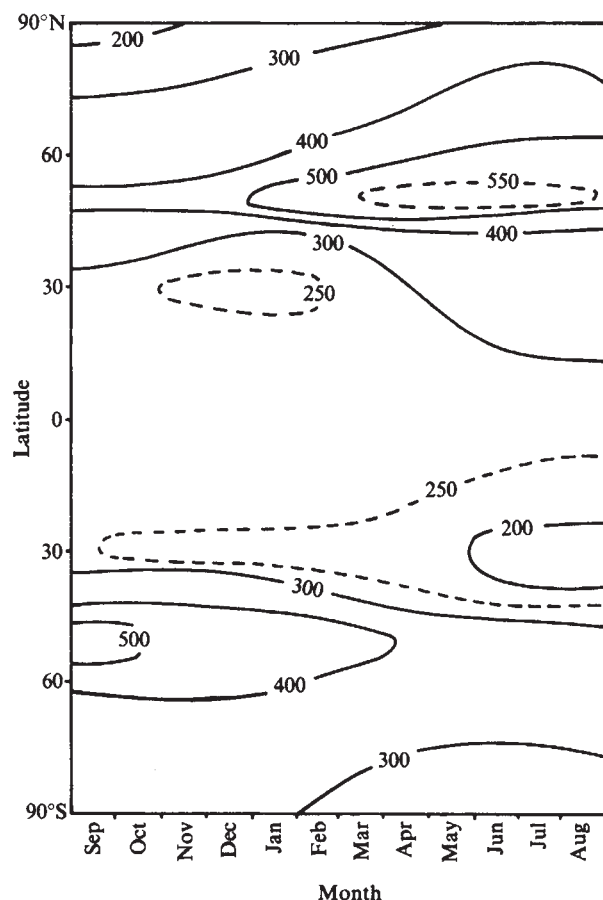


Fig. 3 Latitude-time section of total ozone (m atm-cm) from experiment 2.

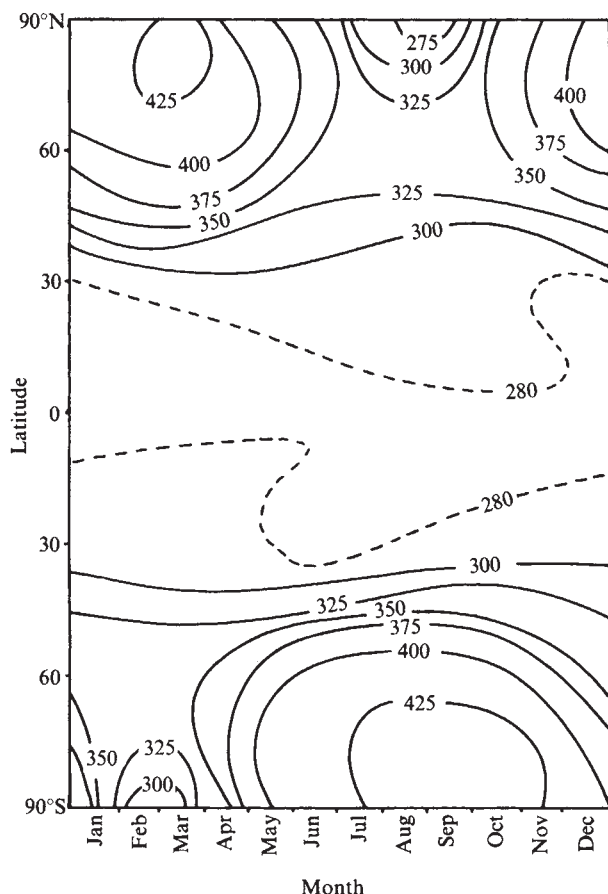


Fig. 4 Latitude-time section of total ozone (m atm-cm) from experiment 3.

The Southern Hemisphere is less well reproduced. Possibly because the eddy activity is less strong in the Southern Hemisphere, the K components should, therefore, be smaller there. Certainly the summer K in both hemispheres should be smaller than used here. In assessing the performance of the model, remember that K ought to vary with the species, whereas here the same K has been used for all species.

The above experiment is crude in that the K values are not calculated within the model. However, the results are encouraging and attempts at a more self-consistent calculation are in progress. An early indication is that the calculated K in winter high latitudes is not sufficient to account completely for the spring build up of ozone and an important role for wave transience is possibly indicated.

An advantage over the usual eulerian models is that the close cancellation between the eddies and the mean motions is removed. In models where the mean circulation is specified, problems of the compatibility of the chosen circulation and the eddy transport coefficients are also partly removed. Another advantage is that experiments with the radiative schemes, including possible perturbation experiments including radiative and hence dynamical feedbacks, are quite simple to perform in this model. Some knowledge of the eddy fields is, of course, still necessary but the model is simpler than the usual eulerian models. Furthermore, note that the cancellation of the eddy terms does not arise when the waves are transient or dissipating. However, because the modelled ozone distribution is quite good it suggests that the chemical eddy contribution may be the most important. Plumb⁸ has shown that the tracer fluxes are oppositely directed during the growing and decaying phases of wave development (see also ref. 14). However, in explosive events like the sudden stratospheric warming we do expect wave transience to play a major part.

Finally, the model explains the success of a similar model by Prabhakara³. His success in a model which used the diabatic circulation was due to the inclusion of a large diffusion term in the ozone continuity equation. With small values of the diffusion coefficients, the behaviour was similar to that shown in Fig. 3. A good ozone distribution was obtained only with the larger values, comparable with the K_{yy} used in experiment 3. The transport modelled by Prabhakara's larger K values was of planetary scale, rather than a sub-grid scale diffusion, and is presumably mainly the chemical contribution discussed above.

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Chloride ions in aqueous solutions

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In spite of much experimental and theoretical work during the past 80 yr, there is at present no generally agreed structural model for concentrated aqueous solutions, even for a relatively simple system like NaCl dissolved in H₂O (ref. 1). Aqueous solutions contain four chemical species and so require 10 pair correlation functions to describe the structure, thus complicating attempts to isolate, for example, ion–water correlations unambiguously from diffraction experiments. The combination of long-range Coulomb potentials, quantum-mechanical hydrogen bonding effects and the intractability of the liquid state has proved an obstacle to theoretical understanding. We have investigated the coordination of water molecules around Cl[−] ions in aqueous solution by the isotopic substitution method. A range of counter-ions and concentrations was used to answer questions about the sensitivity of Cl[−] hydration to the nature of the counter-ion and the ionic strength. We show here that Cl[−] hydration is essentially independent of the cation and of ionic strength with the possible exception of a transition metal counter ion (Ni²⁺). The insensitivity of Cl[−] hydration to cation type should lead to a major simplification in the theory of solutions.

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The unique role of neutron diffraction applied to aqueous solutions has been emphasized by two groups^{2,3}. Narten *et al.*² demonstrated ion-water effects by comparing the neutron and X-ray scattering intensity from solutions of LiCl in D₂O. The Bristol/Leicester group³⁻⁵ emphasized the use of isotopic substitution and showed that the coordination of water molecules around a given ion can be derived unambiguously from the algebraic difference, as a function of momentum transfer, $\hbar k$, of the absolute neutron-scattering cross-sections between two samples identical in all respects except that the isotopic state of the ion has been changed⁴. We have also shown that the recoil corrections which apply to neutron diffraction from aqueous solutions essentially vanish when this difference is used. Using this method the nature of the hydration of Ni²⁺ has been shown⁵ to be concentration dependent, an unexpected result.

We now report on an investigation of the Cl⁻-H₂O coordination (loosely referred to as hydration) in a range of different ionic solutions (Table 1) to establish the extent to which the geometry of ionic hydration is sensitive to the counter-ion. The parameters which define this geometry are shown in Fig. 1. This sensitivity has long been a controversial matter and has not previously been satisfactorily resolved either by theory⁶ or experiment⁷. The experiments were carried out at the ILL Grenoble and a full account of the method has been given elsewhere⁴.

For a given solution, two electrolytes M³⁵Cl_n and M³⁷Cl_n are dissolved in (heavy) water. The isotopic substitution made, ³⁵Cl → ³⁷Cl, changes the Cl scattering length f from 11.7 fm to 3.50 fm. This yields⁴ a first-order difference $\Delta_{\text{Cl}}(k)$ between two sets of diffraction data given by

$$\Delta_{\text{Cl}}(k) = A(S_{\text{ClO}}(k) - 1) + B(S_{\text{ClD}}(k) - 1) + C(S_{\text{ClM}}(k) - 1) + D(S_{\text{ClCl}}(k) - 1) \quad (1)$$

where

$$A = \frac{2}{3}nc(1 - c - nc)f_{\text{O}}(f_{\text{Cl}} - f'_{\text{Cl}})$$

$$B = \frac{4}{3}nc(1 - c - nc)f_{\text{D}}(f_{\text{Cl}} - f'_{\text{Cl}})$$

$$C = 2nc^2f_{\text{M}}(f_{\text{Cl}} - f'_{\text{Cl}})$$

$$D = n^2c^2(f_{\text{Cl}}^2 - (f'_{\text{Cl}})^2)$$

c is the atomic fraction of cations, n is the cation valence and S_{ClO} , S_{ClD} , S_{ClM} and S_{ClCl} represent respectively the partial-structure factors related to chlorine-oxygen, chlorine-deuterium, chlorine-cation and chlorine-chlorine correlations. The numerical coefficients $A \dots D$ are shown in Table 1 and the method exploits the fact that A and B are much larger than C and D . The Fourier transform of equation (1) yields⁴ a weighted radial distribution given by

$$G_{\text{Cl}}(r) = A[g_{\text{ClO}}(r) - 1] + [Bg_{\text{ClD}}(r) - 1] + C[g_{\text{ClM}}(r) - 1] + [Dg_{\text{ClCl}}(r) - 1]$$

where $g_{\alpha\beta}(r)$ is the partial radial distribution function for β ions as seen from an α ion. However, A and B are much greater than C and D and because water is molecular in character, $G_{\text{Cl}}(r)$ gives the distribution of water around the Cl⁻ ion.

The characteristic form for $G_{\text{Cl}}(r)$ has been published previously for solutions of NaCl (ref. 4) and CaCl₂ (ref. 8) and turns out to be typical for all solutions studied. The significant results from these data are presented in Table 2, from which conclusions about the character of Cl⁻ hydration can be drawn.

(1) r_{ClO} falls in the range 3.20 ± 0.04 to 3.34 ± 0.04 Å. In general these distances are greater than those found in X-ray studies⁷—a consequence of the inherently poor discrimination of total X-ray patterns from solutions and the need to analyse the data in terms of a rather simple model⁹. They are in excellent

Table 1 Scattering lengths and sample parameters

Electrolyte solution	Isotopes	Abundance (%)	Scattering lengths (10 ⁻¹² cm)	c	Molality	A (barns) 10 ⁻²	B (barns) 10 ⁻²	C (barns) 10 ⁻³	D (barns) 10 ⁻³
LiCl	³⁵ Cl	99.35	1.17	0.598	9.95	1.66	3.83	-1.34	4.38
	³⁷ Cl	90.4	0.35						
	³⁵ Cl	99.35	1.17	0.023	3.57	0.695	1.60	-0.2	0.6
	³⁷ Cl	90.4	0.35						
NaCl	³⁵ Cl	99.35	1.17	0.0331	5.32	0.84	1.92	0.50	1.20
	³⁷ Cl	90.4	0.35						
RbCl	³⁵ Cl	99.35	1.17	0.0279	4.36	0.82	1.88	1.00	0.90
	³⁷ Cl	90.4	0.35						
CaCl ₂	³⁵ Cl	99.35	1.17	0.0275	4.49	1.60	3.68	1.16	3.77
	³⁷ Cl	90.4	0.35						
NiCl ₂	³⁵ Cl	99.35	1.17	0.027	4.35	1.57	3.63	2.40	3.60
	³⁷ Cl	90.4	0.35						
NiCl ₂	³⁵ Cl	99.35	1.17	0.019	3.00	1.07	2.47	1.10	1.70
	³⁷ Cl	90.4	0.35						
BaCl ₂	³⁵ Cl	99.35	1.17	0.00719	1.10	0.47	1.07	0.10	0.15
	³⁷ Cl	90.4	0.35						

Table 2 Cl-water coordination

Solute	Molality	$r_{\text{ClO}}^{(1)}$ (Å)	r_{ClO} (Å)	$r_{\text{ClD}}^{(2)}$ (range) (Å)	ψ^* (range) (deg)	Coordination no.
LiCl	3.57	2.25 ± 0.02	3.34 ± 0.04	—	0	5.9 ± 0.2
LiCl	9.95	2.22 ± 0.02	3.29 ± 0.04	3.50–3.68	0	5.3 ± 0.2
NaCl	5.32	2.26 ± 0.03	3.20 ± 0.05	—	0–10	5.5 ± 0.4
RbCl	4.36	2.26 ± 0.03	3.20 ± 0.05	—	0–10	5.8 ± 0.3
CaCl ₂	4.49	2.25 ± 0.02	3.25 ± 0.04	3.55–3.65	0–6	5.8 ± 0.2
NiCl ₂	4.35	2.29 ± 0.02	3.20 ± 0.04	3.40–3.50	6–11	5.7 ± 0.2
NiCl ₂	3.00	2.23 ± 0.03	3.25 ± 0.05	—	0–6	5.5 ± 0.4
BaCl ₂	1.10	2.24 ± 0.04	3.26 ± 0.05	—	0–6	6.2 ± 0.4

* Assuming that r_{OD} is 1 Å.

agreement with the recent quantum-mechanical calculation of Clementi *et al.*¹⁰. r_{ClD} spans the range 2.23 ± 0.02 to 2.29 ± 0.02 Å.

(2) The data favour the D-bond configuration (Fig. 1)⁴ but with a tilt angle which is always less than 11° . Clearly the influence of the counter-ion on Cl-hydration is a second-order effect and this explains why it had not been unambiguously identified experimentally. Computer simulations based on the so-called ST2 potential predict significant counter-ion effects. Clearly, this widely used potential function needs critical re-examination and, indeed the alternative 'central force' model is also worth studying¹¹.

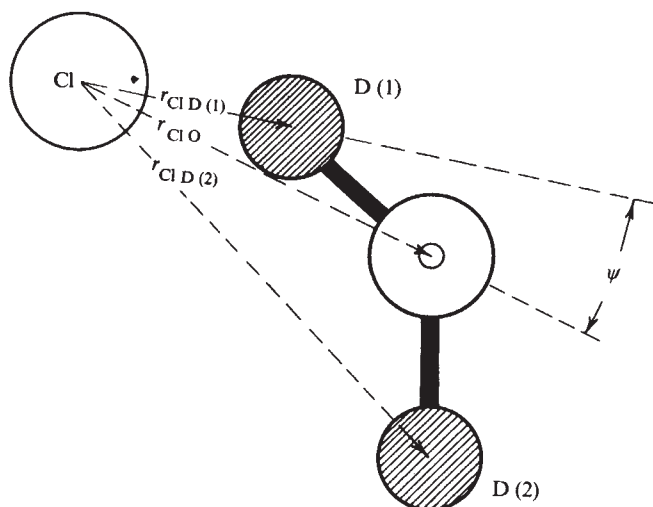


Fig. 1 The geometry of Cl^- hydration.

(3) The experimental uncertainty in r_{ClD} and r_{ClO} is such that all the solutions, except NiCl_2 at high concentrations, could be characterized by $\psi = 0$. Whether the data for Ni^{2+} represent a general transition metal counter-ion effect must await further studies involving Fe^{2+} and Co^{2+} .

(4) The coordination number obtained by integrating G_{Cl} (ref. 2) over the first peak cannot necessarily be identified as a 'hydration' number¹². This is because water molecules are entering and leaving the neighbourhood of a Cl^- ion on a time scale of $\sim 10^{-12}$ s (compared with cation-water residence times which are often $\sim 10^{-6}$ s (ref. 13) and where the equivalence of coordination and hydration is much clearer). Hence the conclusion that the 'hydration number' for Cl^- in solution may be concentration dependent needs care. The dynamical aspects of hydration have to be looked at in detail and such an experiment is in progress (Hewish and Howells, personal communication).

We conclude that the counter-ion effects are sufficiently small to be disregarded in a first-order theory and that quantum-mechanical calculations based on a single Cl^- surrounded by a small number of water molecules are the best guide to ion-water conformations in solution. The possibility that transition metal ions at high concentration may produce measureable counter-ion effects cannot, however, be ruled out.

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Chemical alkylation of lead (II) salts to tetraalkyllead (IV) in aqueous solution

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Methylation of lead in the environment would have serious consequences for water quality and for the well being of aquatic biota. As there is strong evidence that tetraalkylleads, the end products of lead alkylation, are considerably more toxic than lead (II) compounds^{1,2}, the elevated levels of inorganic lead now present in inland waterways and sediments as a result of industrial and motor vehicle emissions will pose a serious environmental hazard if mechanisms exist for the conversion to alkyllead (IV) species in aquatic systems. In the belief that the key to biological Pb(II) methylation lies in methyl transfer to Pb(II) from a carbonium ion donor (for example, *S*-adenosyl-methionine), we recently initiated chemical and biological studies on the reactions of CH_3^+ donors with neutral and anionic Pb(II) compounds. We describe here the unequivocal synthesis of volatile tetramethyllead and other tetraalkylleads from Pb(II) salts and simple chemical reagents in aqueous solution. The known occurrence of methyl iodide in natural waters³ and our demonstration that Me_4Pb is readily synthesized from this reagent and Pb(II) salts in aqueous solution could have far reaching significance not only for the chemical synthesis of toxic organoleads but also for possible mechanisms of microbiological methylation.

Since the initial report by Wong *et al.*⁴ of microbiological conversion of trimethyllead acetate (Me_3PbOAc) and Pb(II) salts into tetramethyllead (Me_4Pb) in active sediments and by pure strains of bacteria, it has been increasingly accepted that trimethyllead salts can be converted to tetraalkylleads via biological mechanisms in addition to disproportionation reactions (refs 5-8 and P. J. Craig and J. M. Wood, personal communication), even though the latter may be catalysed by the presence of sulphide ion⁹. For inorganic Pb(II) , however, several questions concerning the facility and mechanism of the initial step to methylate a Pb(II) species remain unresolved. There are conflicting reports as to whether tetramethyllead can be formed from Pb(II) salts in aqueous systems. A report from Huber's laboratory⁷ claimed formation of Me_4Pb from Pb(II) acetate with microorganisms cultivated in aquarium water, but workers from Ethyl Corporation (J. Bentz, M. Brandt, A. Pallay and G. Ter Haar, personal communication) failed to note increased levels of Me_4Pb above sediments enriched with Pb(II) nitrate. There was no reported reaction between Pb(II) and methylcobalamin in aerobic or anaerobic conditions^{6,9,10}. In contrast, Witman and Weber¹¹ presented evidence for the demethylation of a synthetic B^{12} analogue by $\text{Pb(ClO}_4)_2 \cdot 3\text{H}_2\text{O}$ in 2-propanol, implying synthesis of a transient methyllead (II) species. More recently, Harrison and Laxen¹² observed abnormally high alkyllead/total lead ratios in atmospheric samples in Morecambe Bay, UK, attributing these levels to natural methylation of

inorganic lead in coastal/estuarine mud flats. An indirect pointer to the existence of a biological mechanism for Pb(II) methylation is the observation that 10–24% of fish from natural environments contain elevated levels of Me₄Pb in lipid fractions^{13,14}.

Of the potential mechanisms available for Pb(II) methylation in water, nucleophilic displacement of X⁻ in PbX₂ (where X is, for example, halide, acetate) by CH₃⁻ to generate Me₂Pb(II) which could subsequently disproportionate to Me₄Pb and Pb(0) is rendered unfavourable by the extreme hydrolytic instability of Pb(II) alkyls¹⁵. Electrophilic attack by CH₃⁺ on Pb(II)X₂ (PbX₃⁻) is more attractive but the initial intermediate of the oxidative addition (MePb(IV)X₂)⁺ has also been presumed unstable because no monomethyllead (IV) compounds have been characterized. Subsequent conversion of monomethyllead (IV) to tetramethyllead (IV) could occur either by disproportionation or by methylation by CH₃⁻ donors⁶. In our initial experiments we have attempted to demonstrate the feasibility of tetraalkyllead synthesis from simple carbonium ion reagents.

Table 1 Methylation of Pb(II) salts with methyl iodide in water

Pb(II) salt	Pb as Me ₄ Pb (ng)	
	pH 5–6	pH 13*
Pb(OAc) ₂ ·3H ₂ O	65.4	370.5
Pb(NO ₃) ₂	90.1	176.8
Pb(ClO ₄) ₂ ·3H ₂ O	72.7	261.9
Pb(COO) ₂	2.3	147.2

Reaction conditions: lead salts, 1.0 mmol; water, 200 ml; MeI, 0.1 ml; reaction time, 5 days at 19 ± 1 °C.

* pH was adjusted by addition of 10 ml of 10% NaOH.

Lead (II) acetate (Pb(OAc)₂·3H₂O) (1.0 mmol) was dissolved in 200 ml of deionized water in a 250-ml filtration flask fitted with an aluminium foil-wrapped stopper. Methyl iodide (0.1 ml, >1 mmol) was added and the flask was sealed with Parafilm. After 5 days at room temperature the head space gases above the solution were withdrawn through the side arm by means of a peristaltic pump and transferred to a U-tube containing 3% OV-1 at -70 °C. The trapped sample was then swept into a sensitive GC-AA system¹⁶ for chromatographic separation and analysis. Tetramethyllead was positively identified by comparison with the retention time of an authentic sample and by molecular weight measurement using mass spectrometry. Standard samples consisting of Pb(OAc)₂·3H₂O alone in deionized water and methyl iodide in deionized water were also analysed to eliminate the possibility of artefacts.

The results summarized in Table 1 indicate that methyl iodide can methylate inorganic Pb(II) compounds in aqueous solution to produce various amounts of Me₄Pb. The lesser amount of Me₄Pb produced from Pb(COO)₂ might well be due to the slight solubility of the lead compound. With low concentrations of Pb(OAc)₂·3H₂O (0.001 and 0.002 mmol) in identical experimental conditions, 2.5 ng and 20.4 ng of Me₄Pb were produced, respectively. The amount of Me₄Pb produced in each case was increased when the reactions were carried out in highly basic conditions. A possible role for sodium hydroxide is to remove iodide ion, thus favouring tetraalkyllead formation over alkyllead iodides. In similar conditions, ethyl iodide (0.1 ml) also reacted with Pb(OAc)₂·3H₂O (1 mmol) to produce tetraethyllead (64.5 ng at pH 5–6 and 190.0 ng at pH 13) along with trace amounts of triethylmethyllead (Table 2).

Mixtures of methyl iodide and ethyl iodide in water without added base gave MeEt₃Pb (1.5 ng) and Et₄Pb (12.0 ng) whereas at pH 13 Me₄Pb (1.3), Me₃EtPb (6.7 ng), Me₂Et₂Pb (97.1 ng), MeEt₃Pb (231.4 ng) and Et₄Pb (349.1 ng) were obtained. In these competition experiments the amounts of tetraalkyllead produced thus increased with increasing ethyl substitution

(Table 2). This may reflect different stabilities for initial (RPb^{IV}X_{3-n})⁺⁺ intermediates (R = Me, Et) in the methylation sequence. It is interesting in this regard that the first examples of EtPbX₃ compounds have recently been described¹⁷.

Table 2 Alkylation of Pb(OAc)₂·3H₂O with different alkylating agents in water

Reagents	pH	Lead as tetraalkyllead (ng)				
		Me ₄ Pb	Me ₃ EtPb	Me ₂ Et ₂ Pb	MeEt ₃ Pb	Et ₄ Pb
MeI	5–6	23.2	—	—	—	—
MeI	13*	224.3	—	—	—	—
EtI	5–6	—	—	—	0.2	64.5
EtI	13	—	—	—	14.5	190.0
MeI + EtI	5–6	—	—	—	1.5	12.0
MeI + EtI	13	1.3	6.7	97.1	231.4	349.1
Me ₃ S ⁺ I ⁻	5–6	—	—	—	—	—
Me ₃ S ⁺ I ⁻	13	1.9	—	—	—	—
Me ₃ SO ⁺ I ⁻	5–6	0.1	—	—	—	—
Me ₃ SO ⁺ I ⁻	13	3.4	—	—	—	—
Me ₃ O ⁺ SbCl ₆ ⁻	5–6	0.2	—	—	—	—
Me ₃ O ⁺ SbCl ₆ ⁻	13	0.1	—	—	—	—

Reaction conditions: Pb(OAc)₂·3H₂O, 1.0 mmol; water, 200 ml; MeI, EtI, each 0.1 ml; Me₃S⁺I⁻, Me₃SO⁺I⁻, Me₃O⁺SbCl₆⁻, each 1.0 mmol; reaction time, 5 days at 19 ± 1 °C.

* pH was adjusted by addition of 10 ml of 10% NaOH.

Apart from alkyl iodides, other potential methyl carbonium ion donors react with Pb(II) salts in aqueous solution to generate Me₄Pb (Table 2). Trimethylsulphonium iodide, trimethylsulphoxonium iodide and trimethylloxonium hexachloroantimonate all yielded the volatile lead alkyl in similar conditions despite the precipitation of insoluble lead-containing compounds in these reactions.

Chemically, the synthesis of organoleads from inorganic reagents is traditionally carried out in strictly anhydrous conditions using, for example, Grignard, organolithium or organomercury reagents. We believe our observations that Pb(II) salts in aqueous solution can react with alkyl iodides to produce Me₄Pb to be unprecedented. It is interesting that the occurrence of methyl iodide (3.1 × 10⁻⁷ g l⁻¹) in natural water has been reported³. We are investigating the possibility of chemical tetraalkyllead synthesis with such an environmental level of MeI and studying other mechanisms for the reactions, including catalysis by other metals.

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Kimberlites in the south-west Pacific

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Pipe-like bodies revealed by seismic refraction surveys over the Ontong Java Plateau (OJP), south-west Pacific, have characteristics of volcanic plugs^{1,2}. The oceanic crust is old (Lower Cretaceous, possibly Jurassic) and unusually thick³ (40 km), similar to that of some continental sections. The Island of Malaita, Solomon Islands, at the thinner periphery of the OJP contains several alnöite intrusions with suites of mantle inclusions, similar to those of the diamond-bearing rock, kimberlite, found in continental areas. Pyroxene geotherms calculated from the inclusions show alnöites to have formed in the Earth's mantle in warmer, shallower conditions compared with kimberlites⁴. However, by analogy with continental cratons, I suggest here that the thicker, northwestern parts of the OJP had lower geothermal gradients suitable for the formation of kimberlite pipes. These could be the bodies revealed by seismic prospecting.

The OJP occupies a 1,600 × 800-km area of the Pacific Ocean to the north-east of Bougainville Island (Papua New Guinea) and the Solomon Island chain, and is broadly defined by a water depth of less than 2,500 m (Fig. 1). Marine seismic studies^{1,2} over the OJP have revealed the presence in the ocean-floor sediments of pipe-like bodies (Fig. 2) which Kroenke has suggested may be of igneous origin. They vary up to 2.5 km across³ and show features which are similar to those produced experimentally by gas fluidization, in particular the presence of marginal faults and the development of crudely bedded conduit filling with inward dips and detached subsided blocks³. Such a mechanism operates in narrow vents and fractures within diatreme volcanoes, including diamond-bearing kimberlites, which could be produced phreatomagmatically, that is, by rising hot magma encountering groundwater⁶. Subsequent subsidence (caldera collapse) along concentric fractures may produce displacements up to 1,280 m (refs 7, 8). It is an important process in enlarging the vent and it also gives rise to a shallow crater or maar into which sediments are washed from a surrounding tuff ring. Progressive post-volcanic subsidence over an extended period may lead to deposition of water-laid sediments⁷ ranging up to more than 300 m thick over the diatremes of some kimberlites and related volcanoes⁹⁻¹¹.

The seismic reflection profiles of the pipe-like bodies in the OJP also indicate collapse features (Fig. 2). Furumoto (personal communication) states that "none of these intrusives in the Ontong Java Plateau has penetrated the surface of the ocean floor. There are some cases where the intrusives have reached very close to the surface. In those cases the ocean floor dips downward directly over it, suggesting a collapse crater or depression".

The features are consistent with the idea that the pipe-like bodies are volcanic intrusions of the kind described above, but it is thought that they broke through to the ocean floor and were subsequently covered by pelagic oozes. Post-volcanic subsidence affected the oozes but with diminishing intensity. Several seismic profiles show a contraction of the disturbed zone overlying the pipe and fading displacements towards the top younger sedimentary layers. The long continued post-volcanic subsidence could be due to progressive solution of carbonate, or other soluble minerals, in the pipe tuffs at such times as they are below the ocean carbonate compensation depth (~4,000–5,000 m). There do not seem to be other deep oceanic volcanic centres with which these types can be readily equated (J. R. Cann, personal communication).

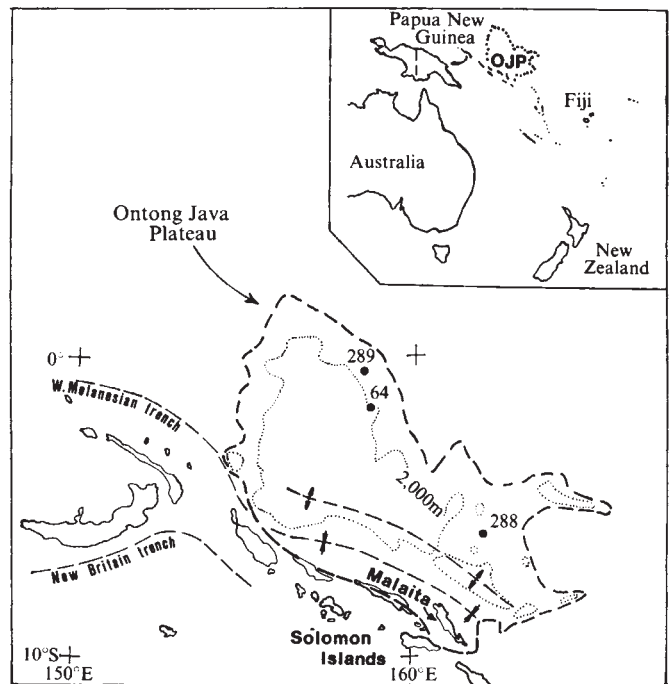


Fig. 1 The Ontong Java Plateau in relation to the islands of the south-west Pacific. The outline follows approximately the 2,500-m isobath, but the buckled southwestern margin is bounded by a thrust^{13,22}. The main north-west trending structures on the OJP are: the Roncador Homocline-Stewart Arch², the North Solomon trench and (not shown) the Malaita Anticlinorium^{2,12,21}. These resulted from collision of the Solomon Islands arc with a westerly moving OJP in late Tertiary times^{16,20}. Deep Sea Drilling Sites 64, 288 and 289 are shown.

Kroenke² has pointed out similarities of the sedimentary succession of the OJP at Deep Sea Drilling Site 64 with that on northern Malaita (Fig. 1) mapped by Rickwood¹². Subsequent mapping^{13,15} and comparison with borehole cores at sites 288 and 289 (ref. 16) confirm that they are ocean-floor deposits ~1,500-m thick. They comprise pelagic foram-nannofossil oozes and chalk down to Oligocene beds, but below this there is an increasing amount of interbedded cherts and siliceous oozes. The oldest sediments encountered are Lower Cretaceous (Aptian). At site 289 and on the island of Malaita and neighbouring Santa Isabel to the north-west, the pelagites overlie, or are interbedded with, ocean-floor tholeiite basalts. These seem to be the topmost representatives of an extremely thick crustal succession of flood basalts which Kroenke¹⁷ suggests mark the beginning of continent formation, that is, the OJP is a proto-continent. The age of the lower members is not known but could extend to the Jurassic.

Furumoto *et al.*^{3,18} have demonstrated a crustal thickness of between 20 and 30 km in the trench and arched region north of Malaita (Fig. 1). The northwestern part of the OJP has an even greater crustal thickness of 35–42 km which is comparable with that of continents, for example, the Colorado Plateau area of North America. This part of the OJP also has a basal layer with a P-wave velocity range of 7.1–7.7 km s⁻¹ immediately overlying the mantle^{3,18}. From this, and the existence of a free air positive gravity anomaly⁴ over the OJP, it has been suggested that burial metamorphism has produced a lower crustal granulite layer similar to that postulated for continents¹⁹.

The southwestern portion of the Ontong Java Plateau, including Malaita, underwent deformation during the Pliocene¹⁴. This resulted in the northwesterly trending folds and the topographic low, the North Solomon trench, which now separate Malaita from the main portion of the OJP². The

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deformation was the response to the collision of westerly moving OJP into a structurally complex Solomon Island arc (Fig. 1) and the more usual subduction process was prevented, or arrested, due to the great thickness of the OJP^{20,21}. The southwestern boundary of the Plateau and its island members—the Pacific Province of P. J. Coleman—is represented by the Korigole thrust²² and associated upthrust mantle ultrabasic rocks^{15,16}. To account for its tectonic behaviour not only the crust but the whole lithosphere of OJP must possess an unusual thickness and rigidity.

Until 1979 only one volcanic centre, at Babaru'u, with possibly a second related intrusion 2 km away, was known²³. Another (Kwaikwai) has since been found and at least three more are indicated by 'shows' of heavy minerals in soils and stream gravels. These minerals include garnets, ilmenites and zircons which can be unusually abundant (Fig. 3). Zircons from two separate localities gave U/Pb ages of 33.9 Myr (ref. 24) and 34.1 Myr (Davis, personal communication). Further prospecting in the dense rain forests will reveal more outcrops.

Although field relationships are uncertain, and no crater sediments have been observed, the alnöites (biotite olivine melilitites) are important as they may be related to the intrusive features in the main part of the OJP described above. The tuffaceous textures and nucleated autoliths²³ are similar to those ascribed to gas fluidization and related phenomena of the olivine melilitite diatremes of the Swabian Alb, Germany²⁶. The rounded, highly polished nature of some of the constituent minerals (Fig. 3) is further evidence of a gas-fluidized origin. From the zircon ages of the alnöites (Lower Oligocene) it is evident that eruptions took place before the folding of the leading edge of the OJP and emergence of Malaita, when the plate was east of its present position.

From stratigraphical considerations it can be seen that anything up to 1,000 m of pelagic oozes could have been laid down after intrusion and before the emergence of Malaita in Plio-Pleistocene times. Although fold movements and erosion have locally revealed the intrusions on Malaita, there is no reason why they should not also occur off-shore. Indeed, this is considered likely in view of the accumulating evidence for increasing numbers of occurrences in northern Malaita itself. There do not seem to have been any seismic reflection traverses in the vicinity of Malaita which could substantiate this claim; most of the

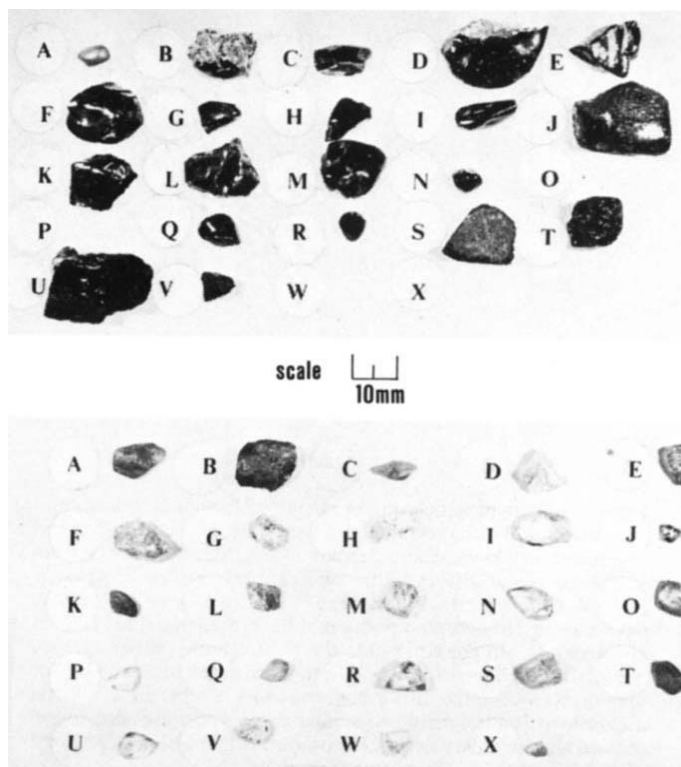


Fig. 3 Ilmenites (top) and zircons (bottom) from an alnöitic intrusion, North Malaita. Their rounded, highly polished nature is attributed to a gas-fluidized emplacement.

pipe-like bodies have been recorded from the northwestern part of the OJP. However, it is this area where the crust is thickest, a factor which could have a profound influence on the kind of volcanicity produced.

Although alnöites are mineralogically and chemically distinct from kimberlites²⁵ they contain similar suites of deep-seated garnet lherzolite (garnet, olivine, orthopyroxene, clinopyroxene) inclusions which give information about the composition and temperature distribution in the mantle at the time of eruption, and also the limiting depth of origin of the host volcanic rock itself^{4,27}. Alnöite seems to have formed at depths²⁵ of ~120–130 km which are shallower than those for kimberlite.

The temperatures and depths of origin of the garnet lherzolites are estimated through the application of experimental and theoretical studies of relevant (Mg-rich) co-existing phases, the Mg orthopyroxene–clinopyroxene solvus for temperature determinations²⁸, and alumina solubility in Mg orthopyroxene for pressure (depth) determinations²⁹ as described by Boyd^{30,31}. More recent experimental data do not significantly affect the results shown in Fig. 4 which are, therefore, used to provide a useful comparison with earlier determined curves from kimberlitic garnet lherzolites. The curves represent the Earth's temperature gradients (palaeogeotherms) below specific intrusions at the time of eruption. The Malaita plots show features which are intermediate between those of 'cool continents', where kimberlites are intruded, and 'hot oceanic' areas. The inflected steeper portion of the geotherms (Fig. 4) has been ascribed to shear heating at the base of lithospheric plate or to diapiric upwelling of asthenospheric mantle (review by Boyd³²). Either interpretation, or combination of both, suggests that the point of inflection is a measure of depth to the base of the lithosphere. The lithospheric thickness calculated by this method in Malaita (southern OJP) is 110 km, which is thinner than that deduced by comparable methods for continents. It is, however, thicker than that in other oceanic areas determined by geophysical methods where values³³ usually average 50–70 km.

Even within a stable continental area (craton) mantle xenoliths from broadly contemporaneous kimberlite pipes indicate

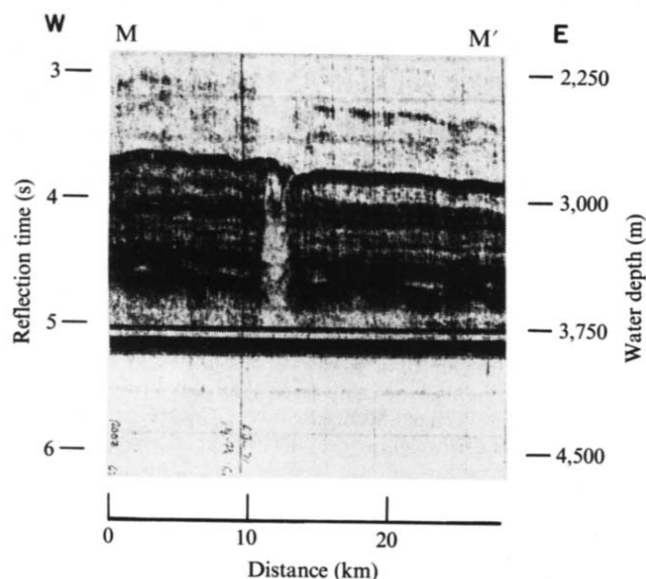


Fig. 2 Seismic reflection profile of a plug-like feature on the Ontong Java Plateau, taken from Kroenke³ (Fig. 19, vertical exaggeration ~12:1. Location about 2°20'S, 161°45'E—slightly outside the OJP boundary as arbitrarily defined in Fig. 1). Note the apparent collapse of bedded vent material.

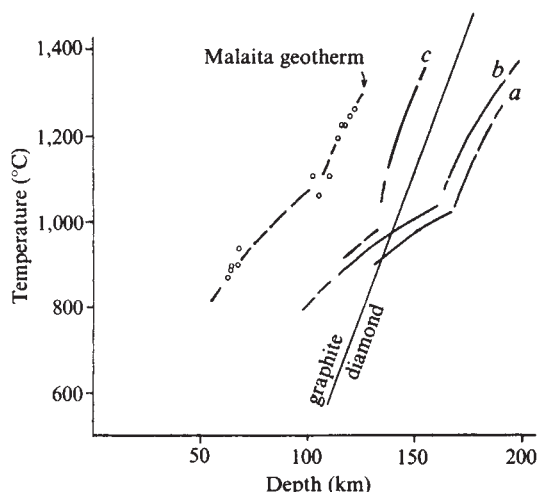


Fig. 4 Malaita palaeogeotherm calculated from temperature and pressure equilibration estimates of garnet ilmenite xenoliths and associated inclusions⁴ compared with those from southern African kimberlite pipes: *a*, from within the Kaapvaal craton near Kimberley³⁴; *b*, from the craton periphery in northern Lesotho³¹; and *c*, from outside the accepted position of the craton boundary in East Griqualand³⁵. In the latter area the palaeogeotherm lies entirely within the graphite stability field³⁶, supporting the observed lack of diamonds within the inclusions and host kimberlites. Olivine melilitite volcanic intrusions, similar to alnöites, are also found outside the boundary of the craton but no palaeogeotherms are available from these.

lateral differences, notably increasingly higher palaeogeotherms (hotter for a given depth) towards the periphery, with an attendant decrease in lithospheric thickness and with lessening probability of diamond formation (Fig. 4) (refs 31, 34, 35).

If the OJP can be regarded as a proto-continent, then by analogy with cratons it could show similar variations in geothermal gradient and lithospheric thickness possibly reflecting the differences in crustal thickness. It follows that the thickest, coolest lithosphere is most likely to be found in the northwestern part of the OJP.

It is suggested that contemporaneously with the Malaita alnöites the thicker northwestern OJP was also intruded by volcanic rocks represented by the pipe-like seismic reflection features. Although these could be alnöites the present evidence favours rocks of a deeper seated provenance, that is, kimberlites. If this is the case then diamonds may exist within the OJP.

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Sedimentological evidence for a stratigraphical break in the Durness Group

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Around Durness north-west Scotland, the top of the Sailmhor Formation, Durness Group, is developed as a palaeokarstic surface deeply penetrated by breccia-filled fissures. Fossil evidence for the age of the Sailmhor Formation is equivocal, but we suggest here that the brecciated surface probably represents a break corresponding to the whole of the Middle and Upper Cambrian. Such a break is also seen in the contemporaneous carbonate successions in Greenland and Spitzbergen.

The limestones and dolomites of the Durness Group include both Lower Cambrian and Lower Ordovician (including Tremadoc or Lower Canadian) fossiliferous beds. It has been held that a continuous sequence of carbonates in north-west Scotland, spanning the whole of this time, is incompatible with contemporaneous deformation and metamorphism of the Dalradian rocks of the Grampian Highlands. As the Dalradian rocks extend from the Precambrian up into the Cambrian system, many authors have considered that the Grampian orogeny must have post-dated formation of the youngest Durness rocks. Strong evidence for the absence of Middle and Upper Cambrian sediments in the Durness sequence will now permit serious consideration of a major orogeny in the Grampian Highlands during this time. This break occurs above all known Lower Cambrian faunas and below rocks which we interpret to be exclusively of Ordovician age. It is not necessary to postulate diagenetic destruction of all Middle and Upper Cambrian fossils.

The Lower Cambrian age for the lowermost Ghrudaidh Formation, is based on the occurrence of *Salterella*^{1–3}. Recently, Lower Cambrian microfossils have been recovered from cherts near the base of the succeeding Eilean Dubh Formation at Inchnadamph, 50 km south of Durness⁴. The ages of the following two formations (Sailmhor and Sangomore) are more doubtful. The lower (Sailmhor) is reported to have produced an age-diagnostic fauna at one locality. The species originally

reported¹ included *Murchisonia* sp., *Euconia* (*Pleurotomaria*) spp., *Cyrotoceras* spp., ?*Orthoceras* sp., *Planolites* and *Asaphus* (*Isotelus*) *canalis*. Doubt has been thrown on the existence of the one specimen of the last-named species, as it is now missing². The rest of the Sailmhor fauna suggests a Lower Canadian (Tremadoc) age². Until now, no body fossils have been reported from the Sangomore Formation which are diagnostic of its age, but a new fossiliferous locality within the Balnakeil Bay outcrop of this unit (NC 3862 6887, see Fig. 1) has yielded *Murchisonia* sp., *Pleurotomaria* sp., and *Orthoceras* sp., suggesting a Lower Canadian (Tremadoc) age. The later formations of the Durness Group (in ascending order, Balnakiel, Croisaphuill and Durine) are of Arenig (and possibly Llanvirn) age^{5,6}.

The absence of Middle and Upper Cambrian calcareous fossils has been attributed to their destruction during the process of dolomitization⁷. It is more likely³ that there is a major stratigraphic break comparable with that which occurs at approximately the same stratigraphical level in the carbonate successions in Ellesmere Island; north-west, north-east and east Greenland; and Spitzbergen^{8,9}.

We have examined the most complete section of the Durness Formation, which is that exposed along the southern shore of Balnakeil Bay (as spelt on the Ordnance Survey New Series 1:50,000 map), west of Durness, north-west Scotland (NC 374688–NC 390687). Rocks from the Eilean Dubh to the Balnakiel Formation are seen here with only minor tectonic disturbance. Our conclusions about the positions of the boundaries between the formations (Fig. 1) correspond to those shown on the 6 inch to 1 mile geological map deposited with the Institute of Geological Sciences in Edinburgh.

The transition between the whiter dolomites of the Eilean Dubh Formation and the darker dolomites of the Sailmhor Formation is gradational and does not suggest a major episode of non-sedimentation. The main changes are an increase in the number of beds with continuous or nodular horizons of chert, and an increase in the number of beds of black and white mottled dolomite. This mottling is almost certainly due to differential dolomitization in branching burrow systems of the *Spongiomorpha paradoxica* type, such as have been described from other Lower Palaeozoic carbonate sequences^{10,11}. In contrast, the junction between the Sailmhor and the overlying Sangomore Formation is sharp. The lowest beds of the latter form a low cliff between NC 3841 6887 and NC 3837 6886. Extending out from the base of this cliff is the upper surface of the Sailmhor. On close examination, it is immediately apparent that the upper part of the Sailmhor Formation is extensively brecciated, whereas this is not so in the base of the Sangomore Formation. The breccias occur, not in planar or lensoid units parallel or sub-parallel to bedding, but as the fillings of vertical fissures. These fissures dissect the Sailmhor Formation, descending from its top down to levels a considerable distance below. They are most abundant in the top 30 m, but at least two of them (Y in Fig. 1) seem to extend right down into the underlying Eilean Dubh Formation, and are, therefore, at least 150 m deep. They range in width from 4 m down to a few centimetres, merging below this range into cracks filled by growth of sparry dolomite. Most are of the order of half a metre to a metre or so wide, and trend in an east–west direction. Others trend north–west–south–east.

The breccias which form the fissure fills consist of angular and sub-angular clasts of dolomite, with subordinate chert fragments. The dolomites appear identical to those forming the beds of the Sailmhor Formation, and many of the fragments show the same burrow mottling. The size of the clasts ranges from sand size up to around 20 cm across. They are very poorly sorted, with the larger fragments being set at all angles in a matrix of the finer material. In some of the fissures, this finer-grained matrix is not present and the voids between the larger clasts has been filled with a crystalline dolomite cement. Towards the bottom of one of the deep fissures which penetrates the Eilean Dubh Formation, the sediment becomes much finer and shows bedding. To establish the density of the fissuring in the plane of bedding, we measured the proportion of fissures to country rock in a 65-m

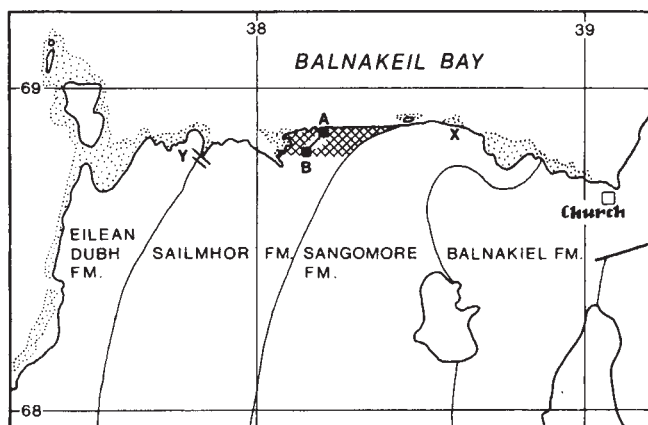


Fig. 1 Map of the south shore of Balnakeil Bay, west of Durness. The boundaries of the formations are after the Institute of Geological Sciences 6-inch geological maps, as modified by T. Robertson. X, Fossil locality; Y, fissures penetrating the top of the Eilean Dubh Formation; A–B transect showing 18.2 m of fissure-fill over a total distance of 65 m. Cross-hatching denotes area of maximum fissure development. Kilometre grid squares are indicated.

transect along a bedding plane about 40 m below the top of the Sailmhor Formation (A–B in Fig. 1); 18.2 m of this transect was accounted for by breccia-filled fissures, and the remainder consisted of undisturbed country rock and country rock with dolomite veins.

In view of the conspicuousness of the fissures with their breccia fills, and the thickness of sediment which they penetrate, why have they not been recognized previously? In fact, the breccias have been noticed, but have apparently always been ascribed to tectonism a long time after deposition. There are local fault breccias, usually of small width, which show displacement of the beds on either side. No vertical displacement at all occurs across the fissures that descend from the top of the Sailmhor Formation. There is also evidence of other sedimentary and diagenetic processes, which produce breccia horizons in the Durness Group as a whole. Young¹² has demonstrated the former presence of evaporite minerals in the Balnakeil Bay section, and locally there are horizontal breccia beds which closely resemble those formed by bed collapse after dissolution of evaporites in the Purbeck Broken Beds of Dorset¹³. Also seen are tepee structures resulting from interstitial cement growth during early diagenesis, and flake breccias which were produced by desiccation¹⁴ and deposited either as horizontal beds or in the quiet micro-environments between stromatolite heads. Furthermore, in most of the other areas in Scotland where the Durness Group is seen (in contrast to the situation around Durness itself), it has been heavily affected by movement along the Moine Thrust zone. In these regions, tectonic brecciation and veining do become prominent. It seems, therefore, that brecciation has previously been regarded as a ubiquitous inconvenience whose effects have to be discounted before the rocks are interpreted.

The junction between the Sailmhor and the Sangomore Formations is well exposed only around Durness. The extensive development of breccia-filled fissures in the Sailmhor Formation is seen in the Balnakeil Bay section, in Durness village itself, and immediately inland. Elsewhere in north-west Scotland, we noted rare, small fissures of a similar type in the upper part of the Eilean Dubh Formation at Kishorn (NG 862443), but we have no way of telling if they originally descended from the top of the Sailmhor Formation.

This extensive and striking development of fissures suggests a major episode of non-deposition during which uplift and extensive erosion of the Sailmhor carbonates occurred. The top of the

formation may be regarded as a major karstic surface. Petrographical details which might throw further light on the process are likely to have been destroyed by the extensive subsequent dolomitization and silicification^{14,15}. The junction between the Sailmhor and Sangomore Formations represents a break corresponding to the Middle and Upper Cambrian. This break justifies the use of the terms Lower Durness Group for the Ghrudaigh, Eilean Dubh and Sailmhor Formations, and the Upper Durness Group for the Sangomore, Balnakiel, Crois-phuill and Durine Formations. The non-sequence seems to correlate with those mentioned above from elsewhere in the Cambro-Ordovician of the North Atlantic region.

Our conclusion seems at first incompatible with the Lower Ordovician (Tremadoc) age assigned to the Sailmhor Formation on fossil evidence¹. However, the diagnostic fauna only comes from a single locality, near the top of the Sailmhor. Its field

relationships are unknown and we have not been able to relocate it. We feel that if this fauna does indeed come from below the Sailmhor/Sangomore boundary, then it represents material from the Upper Durness Group that has fallen into one of the fissures in the Lower Durness Group.

The timing of the Grampian Orogeny is still not definite, but it now seems probable that it occurred before the deposition of the late Tremadoc/early Arenig Lough Nafuoey Group of western Ireland¹⁶. The early Ordovician rocks along the Highland Border (in Arran and near Aberfoyle) are perhaps (together with the Macduff Slates) younger than the main episodes of the orogeny. If this is so, the interval between the Lower Cambrian and Lower Ordovician (Tremadoc) rocks at Durness would correspond in time to the peak of the Grampian Orogeny.

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Palaeoceanographic change in the Pacific at the Eocene-Oligocene boundary

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Oxygen isotopic studies both of benthic foraminifera¹⁻⁵ and shallow-marine carbonates⁶⁻⁸ have provided a useful monitor of marine palaeotemperatures. The Deep Sea Drilling Project (DSDP) has provided cores from many ocean basins to conduct detailed stable isotopic and palaeoceanographic studies of the Cenozoic and late Mesozoic. DSDP Sites 277 and 292, separated by ~60° latitude in Palaeogene times, each record an ¹⁸O enrichment in benthic foraminifera of nearly 1‰ beginning at the Eocene-Oligocene boundary. Planktonic foraminiferal trends are similar to benthic trends in the high latitude southwest Pacific Ocean, but tropical planktonics show only a minor (~0.3‰) increase which may reflect a change in seawater composition. These results suggest a sudden cooling of Pacific deep waters and high latitude surface waters forms a useful stratigraphic marker for the Eocene-Oligocene boundary. This boundary is particularly important because of its association with several worldwide palaeo-oceanographic and biogeographic changes. These include a sudden drop in the calcite compensation depth of 1-2 km (refs 9, 10); a decrease in planktonic microfossil diversity¹¹⁻¹³; a change in planktonic biogeographic patterns¹²⁻¹⁴; and increased erosion of deep-sea sediments over wide areas^{15,16}.

Most studies of the Eocene-Oligocene boundary (Table 1), based on coarse sampling, have revealed an early to middle Eocene temperature maximum in both surface and deep waters followed by cooling through the late Eocene into the early Oligocene. This is manifested as an oxygen isotopic enrichment of ~2‰ (equal to an 8 °C cooling) over a period of about 10 Myr. The temperature record at high latitudes reveals synchronous cooling in both surface and deep waters³ whereas if low and middle latitudes were included the surface waters are shown to cool more gradually⁵. Most studies also record a benthic

foraminiferal oxygen isotopic enrichment of ~1‰ (~4 °C cooling) at or near the Eocene-Oligocene boundary. Only in a detailed study⁴ of this change at DSDP Site 277 is it shown to occur in the earliest Oligocene.

DSDP Site 277 was drilled in 1,214 m water depth on the Campbell Plateau in the south-west Pacific (52° 13.43' S; 166° 11.48' E). The Campbell Plateau is continental in origin¹⁷ and plate tectonic reconstructions place it only a few degrees farther south 38 Myr ago^{18,19}. Site 292 (ref. 20) is located at 2,943 m on the Benham Rise in the west Philippine Sea (15° 49.11' N; 124° 39.05' E). This is a tectonically complex region of anomalously shallow oceanic crust²¹ which has been drifting northward since the early Palaeogene²² so the location of Site 292 was tropical in Eocene-Oligocene times.

Microfossil ranges²³⁻²⁷ of potential stratigraphic value shown in Figs 1 and 2. The biostratigraphic sequence at Site 277 is marked only by last appearances. The Eocene-Oligocene

Table 1 Summary of Eocene to earliest Oligocene stable isotopic studies

Site	Location	Ref.	Micro fossil type*	Bio-stratigraphic zone† in which change takes place	Approximate amount of isotopic enrichment
305	Shatsky Rise	37	N	P10 to P18	1.3
167	Central Equatorial Pacific	37	N	P18 to P19/20	0.9
167	Central Equatorial Pacific	2	B	P16 to P18	1.0
167	Central Equatorial Pacific	5	P	P14 to P17	1.0
44, 171	Central North Pacific	5	B	P16 to P18	1.0
44, 171	Central North Pacific	5	P	P13 to P18	0.5
277	Campbell Plateau	3	B & P	early to late Eocene‡	2.0
277	Campbell Plateau	4	B	earliest Oligocene‡	1.0
357	Rio Grande Rise	38	B	P14 to P17	1.0
357	Rio Grande Rise	38	P	P13 to P14	1.0
398	Off Portugal	39	B	P15 to P16	2.0
398	Off Portugal	39	P	P13/14 to P16	2.0

*B, Benthic foraminifera; P, planktonic foraminifera; N, nanofossils.

†Zones after Blow²³.

‡P zones not applicable.

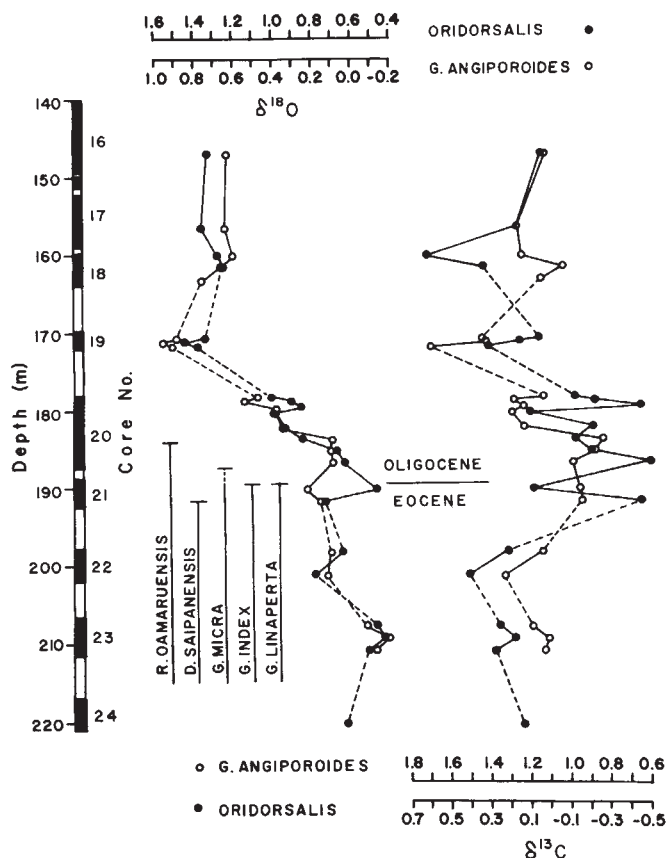


Fig. 1 Stable isotopic results (‰, PDB) at high latitude Site 277 on the Campbell Plateau, South-west Pacific Ocean. Intervals of core recovery are in black along the vertical axis. Microfossil ranges are from refs 23, 24. Benthic and planktonic foraminiferal $\delta^{18}\text{O}$ suggests the entire water column at this location cooled by 3 to 4 °C beginning at the Eocene-Oligocene boundary.

boundary is defined in New Zealand by the last appearances of the planktonic foraminifera *Globigerina index* and *Globigerina linaperta* which are considered²⁸ to occur within planktonic foraminiferal zone P17. Nannofossil markers²⁴ place the Eocene-Oligocene boundary within a few metres of that defined by planktonic foraminifera (Fig. 1).

Although there are first and last appearances of taxa throughout the latest Eocene and earliest Oligocene at Site 292, several last appearances suggest that the Eocene-Oligocene boundary lies within core 35 (Fig. 2). Ujiie²⁵ places the boundary at the last appearance of the foraminifera *Globorotalia cerroazulensis* and *Hantkenina primitiva* (approximately equivalent to the top of zone P17). The base of zone P17 is defined by the last appearance of *Cribohantkenina inflata*²⁸, so core 35 of Site 292 is probably time-equivalent to core 21 of Site 277. Ellis²⁶ finds important nannofossil markers (last appearances of *Discoaster barbadiensis* and *Discoaster saipanensis*) 8–13 m lower in the section. The presence of the short ranging latest Eocene radiolarian *Lophocyrtis jacchia* suggests that the sequence is nearly complete at this location²⁷.

Isotopic analyses followed standard procedures²⁹. B-1 was used as a working standard and was calibrated to PDB using NBS-20 (Solnhofen limestone) as an absolute standard. For NBS-20 we take $\delta^{18}\text{O} = -4.18\text{‰}$ and $\delta^{13}\text{C} = -1.07\text{‰}$ with respect to PDB³⁰. Eleven analyses of NBS-20 have been performed giving $\delta^{18}\text{O}$ B-1 = $-4.12 \pm 0.12\text{‰}$ (1 σ of the isotopic difference of samples run on a single day) and $\delta^{13}\text{C}$ B-1 = $-1.71 \pm 0.06\text{‰}$. As a check we have run 10 samples of the standard TKL-1, giving $\delta^{18}\text{O}$ PDB = $-4.43 \pm 0.10\text{‰}$ and $\delta^{13}\text{C}$ = $-1.78 \pm 0.28\text{‰}$ which compare well with the mean isotopic compositions and standard deviations determined by 15

different laboratories of $-4.26 \pm 0.18\text{‰}$ and $-1.69 \pm 0.13\text{‰}$ for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ respectively³⁰. We have also run 11 Yule Marble samples as part of the CENOP intercalibration, giving $\delta^{18}\text{O} = -6.63 \pm 0.08\text{‰}$ and $\delta^{13}\text{C} = -2.30 \pm 0.05\text{‰}$ which compare well with -6.49 , -2.28 and -6.57 , -2.30 of J. Killingley (personal communication) and S. Savin (personal communication) respectively.

The benthic foraminifera *Oridorsalis* was chosen from the size fraction greater than 175 μm at both locations. There was no single abundant, long-ranging species of planktonic foraminiferal fauna common to each site that could be used for the isotopic analysis. Thus, *Globigerina angiporoides* was selected from Site 277 and *Globigerina ampliapertura* from Site 292. Both are from the 175–295 μm size fraction. I have assumed that both of these planktonic species previously inhabited near-surface waters and thus their isotopic composition mostly reflects variation in surface-water temperature.

Table 2 Stable isotopic results (‰, PDB) of the present study

Sample	Depth (m)	Site 277 <i>Oridorsalis</i> sp.		<i>Globigerina angiporoides</i>	
		$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$
16-5, 140	147.4	1.33	0.05	0.63	1.11
17-5, 140	156.9	1.36	0.18	0.64	1.25
18-1, 140	160.4	1.28	0.63	0.60	1.23
18-2, 140	161.9	1.25	0.34	0.66	1.02
18-3, 140	163.4			0.76	1.13
19-2, 100	171.0	1.34	0.06	0.89	1.43
19-2, 140	171.4	1.45	0.16	0.96	1.41
19cc	172.0	1.38	0.32	0.91	1.69
20-1, 50	178.5	1.00	-0.12	0.47	1.12
20-1, 100	179.0	0.90	-0.22	0.54	1.27
20-2, 19	179.7	0.85	-0.45	0.38	1.22
20-2, 100	180.5	0.99	0.11	0.38	1.28
20-3, 140	182.4	0.93	-0.21	0.34	1.22
20-4, 140	183.9	0.84	-0.12	0.09	0.82
20-5, 140	185.4	0.67	-0.20	0.10	0.86
20-6, 140	186.9	0.63	-0.50	0.09	0.97
21-2, 140	190.4	0.47	0.09	0.22	0.94
21-3, 140	191.9	0.73	-0.45	0.15	0.93
22-1, 140	198.4	0.64	0.22	0.10	1.13
22-3, 140	201.4	0.78	0.42	0.12	1.32
23-1, 140	207.9	0.47	0.27	-0.08	1.18
23-2, 140	209.4	0.43	0.19	-0.19	1.10
23-3, 145	211.0	0.51	0.29	-0.13	1.12
24-3, 140	220.4	0.62	0.15		
Site 292					
32-1, 100	292.50	1.60	0.44	-1.23	1.39
32cc	293.50	1.75	-0.12	-1.11	1.32
33-1, 24	301.24	1.61	-0.13	-1.18	1.39
33-2, 50	303.00	1.74	0.11	-1.07	1.57
33-2, 100	303.50	1.83	0.11	-1.16	1.40
33cc	304.50	1.98	0.51	-1.04	1.95
34-1, 52	311.02			-1.41	1.75
34-1, 99	311.49			-1.02	1.73
34-1, 148	311.98	1.92	0.62	-1.34	1.95
34-2, 50	312.50			-1.27	2.09
34-2, 99	312.99			-1.03	2.16
34-2, 148	313.48	2.26	0.56		
35-1, 87	320.87	1.04	0.23	-1.21	1.75
35-1, 99	320.99	0.95	0.26	-1.29	1.75
35-1, 141	321.41	1.20	0.23	-1.61	1.50
				-1.39	1.61
35-2, 36	321.86	0.85	0.48	-1.66	1.50
35-2, 101	322.51	1.01	0.25	-1.01	1.56
35-2, 141	322.91	0.96	0.01		
35-3, 95	323.95	0.71	-0.31	-1.52	
35-3, 147	324.47			-1.32	1.30
				-1.29	1.27
36-1, 89	330.39	0.87	-0.01		
36-1, 145	330.95	0.78	-0.10		
36-2, 59	331.59	0.82	-0.21	-1.42	1.43
36-2, 89	331.89	0.66	-0.25	-1.50	1.46
36-3, 117	333.67	1.04	0.09	-1.26	1.44
36-3, 136	333.86			-1.58	1.33
36-4, 116	335.16			-1.57	1.44
36-5, 85	336.35	0.92	0.03		
37-1, 88	339.88	1.01	0.24		
37-2, 100	341.50	1.40	0.24		
37-2, 148	341.98	1.01	0.08		
37-3, 50	342.50	1.06	0.23		

Benthic foraminiferal calcite becomes enriched in ^{18}O at both Sites 277 and 292 beginning at the Eocene–Oligocene boundary (Figs 1 and 2). At high southern latitude Site 277, the change in $\delta^{18}\text{O}$ between 170 and 190 m is about 1‰, whereas the average difference before and after the transition is slightly less (0.8‰). At Site 277 the planktonic and benthic $\delta^{18}\text{O}$ records are highly correlated. Site 292 also shows a large change in benthic $\delta^{18}\text{O}$ which is between 312 and 320 m, and is greater than the average difference below and above the transition (about 1.5 versus 0.9‰). The most important difference between the two sites is that unlike the high latitude site, at tropical Site 292 the planktonic foraminifera become gradually enriched in ^{18}O by about 0.3‰ over the interval examined. There is no abrupt change in Site 292 planktonic foraminiferal $\delta^{18}\text{O}$ at the Eocene–Oligocene boundary, but the average above and below the benthic transition are significantly different (0.28‰).

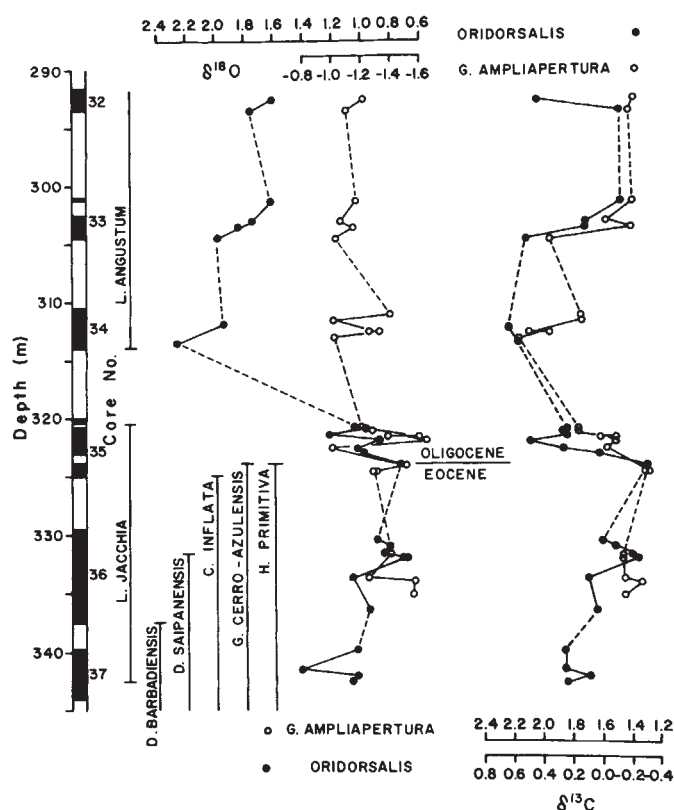


Fig. 2 Stable isotopic results (‰, PDB) at tropical Site 292 in the Philippine Sea. Intervals of core recovery are in black along the vertical axis. Microfossil ranges are from refs 25–27. Only benthic foraminiferal $\delta^{18}\text{O}$ suggests a 3 to 4°C cooling beginning at the Eocene–Oligocene boundary. The slight enrichment in planktonic foraminiferal ^{18}O may record a minor change in seawater composition.

The $\delta^{13}\text{C}$ records of benthic and planktonic foraminifera appear subparallel at both locations (Figs 1 and 2). At Site 277 there is evidence that foraminiferal carbon across the Eocene–Oligocene boundary is lighter on average by a few tenths of a permil than before or after. Site 292 is marked by isotopically heavier foraminiferal carbon in the earliest Oligocene.

The oxygen isotopic results agree with an abrupt earliest Oligocene enrichment⁴ in benthic foraminiferal ^{18}O (Figs 1, 2). My results differ from those of Kennett and Shackleton⁴ for Site 277 in that the isotopic enrichment begins at the Eocene–Oligocene boundary and the transitional change is less by 0.2‰. This is due to my use of monogeneric samples (*Oridorsalis* sp.) compared with mixed benthics of Kennett and Shackleton⁴. This

is important because the oxygen isotopic composition of different benthic foraminifera from the same sample may vary by nearly 1‰ (refs 31, 32). I have also used a closer sampling interval, especially in core 20; plotted core 19 at the top of the cored interval rather than the bottom; and used Jenkins²³ range of *G. index* rather than that of the Site Report, Site 277 (ref. 17). The more accurate placement of the last appearance of *G. index* raises the boundary about 2 m from 192 to 189.6 m. I have checked samples between the two reported last appearance levels of *G. index* (21–2, 60 and 21 cc) and found it present in 21–2, 140 but absent from 21–3, 140. Thus its uppermost range is sporadic and partly leads to uncertainty in placement of the Eocene–Oligocene boundary.

The maximum duration of the oxygen isotopic transition may be estimated from published sedimentation rates. At Site 292 the late Eocene to early Miocene sedimentation rate is $\sim 12\text{ m Myr}^{-1}$ (ref. 20), so the interval from the middle of core 35 to the base of core 34 represents a maximum duration of about 0.9 Myr. The sedimentation rate of Site 277 above core 21 is $\sim 22\text{ m Myr}^{-1}$ (ref. 17). The $\delta^{18}\text{O}$ increase occurs over a maximum interval of $\sim 18\text{ m}$, giving a maximum duration of about 0.8 Myr, in good agreement with Site 292.

Three mechanisms which may account for the observed oxygen isotopic records, either singly or combined, are post-depositional alteration, a change in the isotopic composition of seawater, or a change in surface and bottom-water temperatures. Taken alone, the $\delta^{18}\text{O}$ record at Site 277 might be suggestive of diagenetic change or seawater compositional change because both benthic and planktonic foraminifera respond in the same way. Diagenetic effects at both sites are ruled out by scanning electron microscopic studies of foraminifera which show the fauna is well-preserved at both sites. Preservation of nannofossils is mostly 'moderate' between cores 16 and 25 at Site 277 (ref. 24), and although there are intervals of poor preservation at Site 292 (ref. 26), they bear no relation to the isotopic change.

A seawater compositional change could explain the shift in isotopic values of both planktonic and benthic species at Site 277, but Site 292 results argue against this. At the tropical location the benthics become markedly enriched in ^{18}O during the earliest Oligocene but the planktonics do not (Fig. 2). This suggests that the maximum seawater compositional change is the maximum enrichment in ^{18}O common to planktonics and benthics at both locations, $\sim 0.3\text{‰}$ between cores 32 and 36 at Site 292. Nevertheless, warming of tropical surface waters at the same time as a large oceanic enrichment in ^{18}O could produce the observed results and cannot be ruled out. Growth of continental ice is a possible mechanism of increasing $\delta^{18}\text{O}$ by 0.3‰. Evidence for Eocene–Oligocene continental ice in Antarctica is equivocal and is discussed by Mercer³³. The sea level record based on seismic stratigraphy indicates a small Eocene–Oligocene regression relative to other Cenozoic changes³⁴. If the ^{18}O enrichment in Site 292 planktonic foraminifera reflects an ice volume growth then the effect was to lower sea level by a few tens of metres.

Most oxygen isotopic studies of Cenozoic marine carbonates follow the interpretation that major Antarctic ice growth began in the middle Miocene, so pre-middle Miocene $\delta^{18}\text{O}$ values from well-preserved foraminifera essentially reflect seawater temperature³. Planktonic microfossils provide evidence of dramatic cooling from the Eocene to the Oligocene. Lipps¹¹ noted that, relative to the Eocene, Oligocene microfossil assemblages were marked by abundant cosmopolitan species of simple morphology. Subsequently Kennett¹² found planktonic microfossil diversity in the Southern Ocean during the Oligocene to be the lowest of the entire Cenozoic. Low faunal and floral diversity and abundant cosmopolitan species are inferred to reflect low marine temperatures in the earlier Cenozoic, as they do today.

If $\delta^{18}\text{O}$ changes are assumed to reflect only temperature changes rather than oceanic compositional changes, then the isotopic results of this study may be used to estimate latest

Table 3 Comparison of foraminiferal $\delta^{18}\text{O}$ data before and after earliest Oligocene cooling

	Benthic foraminifera			Planktonic foraminifera			Vertical isotopic contrast (%)
	<i>n</i>	$\bar{x}$ (%)	σ	<i>n</i>	$\bar{x}$ (%)	σ	
Early Oligocene							
Site 277 (cores 16–19)	7	1.34	0.07	8	0.76	0.14	0.58†
Site 292 (cores 32–34)	8	1.84	0.22	10	−1.16	0.13	3.00†
latitudinal contrast					1.92*		
Late Eocene							
Site 277 (cores 21–24)	8	0.58	0.13	7	0.03	0.16	0.55†
Site 292 (below 35–3, 95)	10	0.96	0.20	6	−1.44	0.13	2.40†
latitudinal contrast					1.47*		

* Computed by subtracting Site 292 $\bar{x}$ from Site 277 planktonic foraminiferal $\bar{x}$.

† Computed by subtracting planktonic foraminiferal $\bar{x}$ from benthic foraminiferal $\bar{x}$.

Eocene and earliest Oligocene latitudinal temperature contrasts, and surface to deep-water temperature contrasts at a particular location (Table 3). Such reconstructions must assume that planktonic foraminiferal $\delta^{18}\text{O}$ largely reflects near-surface water temperature. It remains to be confirmed where *G. anguloroides* lived in the water column. An isotopic study of Oligocene planktonic foraminifera suggested *G. ampliapertura* inhabited intermediate-depth waters³⁵. However, as *G. ampliapertura* does not show an earliest Oligocene $\delta^{18}\text{O}$ increase similar to the benthic foraminifera, I assume that its isotopic composition mostly reflects near-surface conditions.

Table 3 summarizes oxygen isotopic data from before and after the earliest Oligocene cooling. The vertical oxygen isotopic contrast between near-surface waters and the sea floor remained the same throughout the late Eocene and early Oligocene at Site 277, so it seems the entire upper water column chilled. At the lower latitude site (292) the vertical $\delta^{18}\text{O}$ contrast increased from 2.4 to 3.0‰, equal to an increase in the shallow to deep temperature contrast of ~2.4 °C. This increase is inferred to be mostly due to a temperature drop in deeper waters⁴. The latitudinal $\delta^{18}\text{O}$ contrast in near-surface waters increased from 1.5‰ in the late Eocene to ~1.9‰ in the early Oligocene, equal to a cooling of ~1.6 °C. This is due to a nearly 3 °C surface-water cooling in high latitudes, while low latitude near-surface waters cooled by only ~1 °C.

Two triggering mechanisms are proposed for the Eocene–Oligocene boundary event. Kennett and Shackleton⁴ interpret the benthic foraminiferal oxygen isotopic enrichment to represent the sudden evolution of cold, thermohaline circulation (the 'psychrosphere'), perhaps in response to the opening of the Tasman Seaway and subsequent sea-ice accumulation around Antarctica. Thierstein and Berger³⁶ argue that this event may have been caused by the injection of water of greatly different density than normal seawater from an isolated basin to the open sea. In particular they propose that brackish water from a previously isolated Arctic Ocean rapidly spread across the surface of the world ocean and disrupted the heat exchange between the deep and surface waters. This is believed to have reduced the moderating influence of the ocean on climate, resulting in snow accumulation on Antarctica, northward migration of the polar front, and the subsequent production of cold bottom water.

The latter hypothesis³⁶ proposes Arctic tectonic events (opening of the Greenland Sea passage) as the trigger of the Eocene–Oligocene cooling rather than the opening of the passage over the Tasman Rise⁴. Thierstein and Berger³⁶ stress the importance of carbon isotopic data: their 'injection event' hypothesis predicts isotopically light carbon in benthic foraminifera at the boundary event and may be tested by detailed studies. At Site 277 benthic carbon is indeed lighter across the boundary (Fig. 1) but it is not so at Site 292 (Fig. 2). Data from more cores are necessary to evaluate mechanisms for the Eocene–Oligocene boundary cooling and the significance of $\delta^{13}\text{C}$ variability.

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Manganese nodule growth rates determined by fossil diatom dating

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We report here that a manganese nodule from the Central Pacific manganese nodule province has been dated by fossil diatoms found in mud scraped from near the nodule's centre. The nodule was taken at DOMES Site B from Core B55-56 at 11°50.3'N and 137°28.2'W in a water depth of 4,892 m. It was resting on the sediment surface, with about 1.5 cm of the nodule bottom (of a total nodule height of 4.2 cm) buried in the mud. The top surface of the nodule was covered with a smooth manganese coating, but the bottom had a very rough, crusty texture. It was found that recent mud had leaked in through cracks in the nodule bottom, but that there were no pre-Pleistocene diatoms in this material. The date obtained was compared with the growth rate determined by the ²³⁰Th excess method and found to be in reasonable agreement. This study adds to the work of Harada^{1,2} on the biostratigraphy (mainly coccoliths) of manganese nodules.

Figure 1 shows the results of the ²³⁰Th dating of the nodule; these are consistent with growth rates found by others using this method for other nodules^{3–5}. The nodule's top 3 mm indicate a growth rate of 3 mm Myr⁻¹, and the bottom yielded a rate of

4.5 mm Myr⁻¹. (The bottom result is only an approximation as the ²³⁰Th excess was quite low.) From the nodule's radius (normal to the sampled section) of 2.7 cm, and assuming a constant growth rate, an age of 9 Myr BP is suggested as the time of initial growth. However, we are aware that the constancy of growth has been questioned⁵.

Two mud samples (B and C in Fig. 2) were taken from the nodule and examined for diatoms. One sample was from the nodule's centre and the other from 0.5 cm below the centre. Both samples contained many fragments of robust, well silicified diatoms. Essentially the only form that was identifiable was *Actinocyclus ingens* Rattra. This form is found sporadically in the early Miocene but is most common in the equatorial Pacific in the early middle and middle middle Miocene (~12–15 Myr BP, close to the ²³⁰Th age). We also encountered occasional specimens (<1%) of the diatom *Pseudoeunotia doliolus* (Wall.) Grunow, an exclusively Pleistocene form.

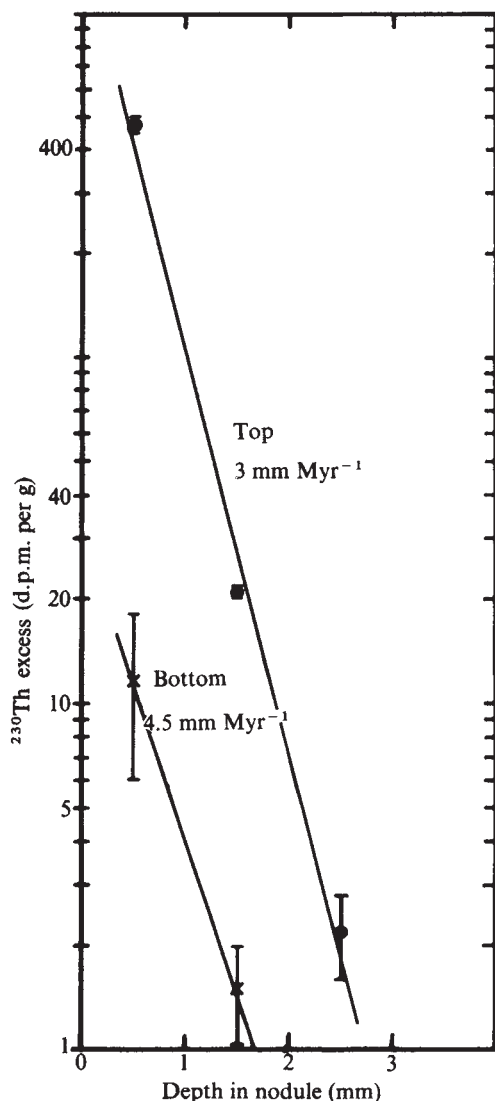


Fig. 1 A log-linear plot of ²³⁰Th excess as a function of depth from the nodule's top and bottom surfaces.

the Matuyama Reversed Epoch (Pleistocene)⁶. Negative evidence, that is, the absence of such forms as *Rhizosolenia praebergonii* var. *robusta* Burckle, *Nitzschia reinholdii* Kanaya and Koizumi, or the silicoflagellate *Mesocena quadrangula* Bukry, suggests that this interval is younger than the Brunhes/Matuyama magnetic boundary. *A. ingens* was not present through this interval nor were there any other pre-Pleistocene forms.

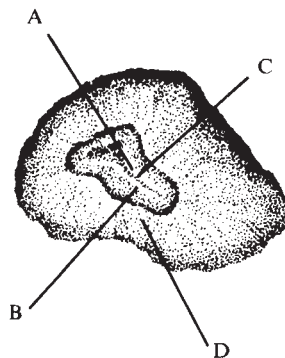


Fig. 2 Schematic diagram of the manganese nodule studied, with sample locations (see text).

Quinterno and Theyer⁷ found several per cent of reworked Tertiary forms of radiolarians in surface Quaternary sediment of nearby cores. However, the percentage of reworked material varied markedly from core to core in their study, and in this core, to a depth of 9 cm, less than 1% of any pre-Pleistocene radiolarians were found. The complete absence of pre-Pleistocene diatoms in this interval is therefore not surprising, as diatoms are much more susceptible to corrosion than are radiolarians.

Mud within the nodule was also analysed for ²³⁰Th. Figure 2 shows the sample locations of the mud taken from the nodule. The sample marked 'D' was 1 cm from the nodule bottom and had a ²³⁰Th concentration of 150 d.p.m. per g. This corresponds to surface sediment and clearly shows that recent mud has leaked in through bottom cracks, and accounts for the presence of the few recent diatoms found in the nodule. Sample 'A', taken from the nodule centre, had only 44 d.p.m. per g, which indicates a mixture of older material with the surface sediment.

The ²³⁰Th analysis and diatom data suggest that surface sediment with high ²³⁰Th (but no Miocene fossils) reached the nodule's interior. The crusty nodule bottom, immersed in sediment, did have cracks where this material could have leaked in. However, material much older than surface sediment is at the nodule centre, and fossil diatoms there can indicate the approximate age of the nodule. This age should not be affected by contamination from recent sediment that probably leaked in through cracks at the nodule bottom.

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To determine if the Miocene diatoms represented contamination from surface sediment, samples from the mud surrounding the nodule were also analysed for diatom species. The diatoms from 0–11 cm depth were representative of Pleistocene or Recent assemblages. The presence of *P. doliolus* indicates that this interval is younger than the Olduvai Event of

Deacidification in a plant with crassulacean acid metabolism associated with anion-cation balance

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In plant tissues using crassulacean acid metabolism (CAM), malate accumulates during the dark and declines in the subsequent light period. The malate is an intermediate store of CO_2 which is fixed photosynthetically during the subsequent day and for CAM plants the amplitude of the fluctuation is significant for plant carbon assimilation¹. Because they are so considerable, the maximum values for acid content aroused early comment². The maximum seems to be related not just to the rate of acidification but also to internal factors which may include the water and cation content of tissues³. Changes in the distribution of cations have recently been linked to the diel fluctuation in malate⁴. I report here that the malate measured in leaves at the end of the day can be correlated with an imbalance between total cations and the organic acids other than malate in the leaves of *Kalanchoe daigremontiana*. There occurs a greater imbalance in leaves of plants grown in a long compared with a short day and this may explain the finding that leaves contain considerably more malate at the end of a long photoperiod.

K. daigremontiana was grown in Long Ashton culture solution until the plants were 9–12 weeks old as described previously³. The macronutrient composition of the solution was 4 mM KNO_3 , 4 mM $\text{Ca}(\text{NO}_3)_2$, 1.5 mM MgSO_4 and 1.33 mM NaH_2PO_4 . Although this supplied nitrogen largely as nitrate, the solution contained 1.86 mM ammonium added as hydroxide to bring its initial pH to 6.2. Plants were grown at 25 °C with either 16 h dark, 8 h light (short day); 8 h dark, 16 h light (long day); or 8 h dark, 8 h low light, 8 h light (pseudolong day). Light was provided by fluorescent and incandescent sources giving an intensity of $0.2 \text{ mE m}^{-2} \text{ s}^{-1}$ at midplant height, except during the period of low light in pseudolong days when the intensity was reduced to $<0.02 \text{ mE m}^{-2} \text{ s}^{-1}$. Leaves were removed at the end of the light period, divided in half and the midrib discarded. From one half organic acids were extracted and measured by gas chromatography⁵. The adjacent half was homogenized and centrifuged at 4,000g for 5 min. The pH of the supernatant was measured. The supernatant and pellet were separated and the pellet rinsed once with 25 cm³ distilled water. After centrifugation at 40,000g for 20 min the supernatant was combined with the original supernatant and assayed for calcium, magnesium, potassium and sodium by atomic absorption spectrophotometry. This extraction consistently removed 99% of the monovalent and 90% of the divalent cations and allowance was made for this loss in calculations of the total cation concentrations.

The photoperiod markedly affected whether malate or isocitrate was the major component of the acid fraction of leaves at the end of the day (Table 1). In long days malate exceeded the sum of isocitrate and citrate whereas in pseudolong and short days the reverse was true. These differences occurred mainly because of large differences in the amount of malate. At the end of a long day leaves contained nine times more malate than after a short day. Several reports show that malate is at or close to its minimum during the diel fluctuation at the end of the photoperiod^{6,7}. Clearly long days do not necessarily lead to low levels of malate in leaves by the end of the day.

Figure 1 shows that increasing amounts of malate occurred together with an increased excess of cations over the anions

Table 1 Amounts ($\mu\text{equiv. per g leaf water}$) of malate, citrate and isocitrate and the pH of extracts of leaves from node 7 (upper) and node 5 (lower) of *K. daigremontiana* analysed at the end of the photoperiod

	Malate	Citrate	Isocitrate	pH
Long day				
Upper	88.2 $\pm$ 2.7	18.5 $\pm$ 3.8	36.3 $\pm$ 4.9	5.1 $\pm$ 0.05
Lower	87.2 $\pm$ 1.9	23.7 $\pm$ 4.9	43.8 $\pm$ 8.1	5.0 $\pm$ 0.05
Pseudolong day				
Upper	46.2 $\pm$ 6.0	26.0 $\pm$ 1.5	53.1 $\pm$ 5.3	5.9 $\pm$ 0.01
Lower	26.4 $\pm$ 0.8	32.0 $\pm$ 3.0	95.0 $\pm$ 8.8	6.0 $\pm$ 0.05
Short day				
Upper	9.4 $\pm$ 2.8	18.5 $\pm$ 0.8	70.1 $\pm$ 4.3	5.9 $\pm$ 0.01
Lower	7.8 $\pm$ 1.8	20.5 $\pm$ 1.0	68.1 $\pm$ 4.5	5.8 $\pm$ 0.02

Results are shown as mean $\pm$ s.e. of at least three replicates.

citrate and isocitrate. The slope of Fig. 1 is very close to unity. It is likely that virtually all the cations have been measured but the organic acids will represent only a component of the total anions in leaves. The intercept in Fig. 1 may provide an estimate of these unmeasured anions in which case this fraction is similar in all leaves at $64.1 \mu\text{equiv. g}^{-1}$. A correlation like that shown in Fig. 1 would occur if *K. daigremontiana* deacidified to a limit determined by a particular balance between total anions and cations in the leaves.

The pH values recorded for leaf extracts were at least 5.0 and in most cases nearly 6.0 (Table 1). The published pK values for malate, citrate and isocitrate indicate that complete dissociation of these acids occurs at about pH 6.0 (ref. 8). The addition of small amounts of alkali to the extracts resulted in a rapid increase in their pH. Thus, by the end of the photoperiod, leaves have deacidified such that there is just sufficient malate to maintain electroneutrality in the leaves by organic acids anions rather than hydroxyl anions. This is very similar to the observation that higher plants generally redress any imbalance between inorganic cations and anions with organic acid, particularly malic acid⁹.

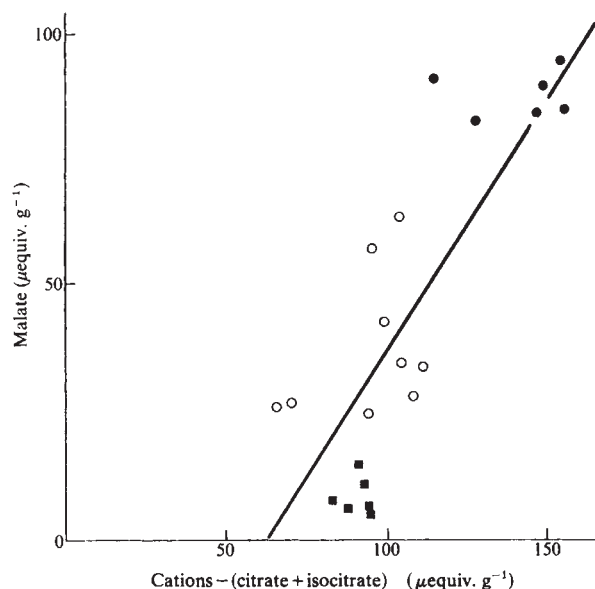


Fig. 1 The amount by which equivalents of cations exceeded citrate and isocitrate in leaves of *K. daigremontiana* and the equivalents of malate in the same leaves measured at the end of three different photoperiods. ●, Long day; ○, pseudolong day; ■, short day. $r = 0.81$, $P > 0.001$; $y = 1.02x - 65.4$.

The results presented here suggest that the malate which fluctuates in CAM plants includes a component which is involved in charge balance and this fraction is not available for CAM.

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Haplodiploid sex ratios and the mutation rate

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Deviations from a 1:1 sex ratio in species with haplodiploid genetics are well known and sociobiologists have produced various explanations^{1–8}, all of which ignore the effect of mutations on haploid viability. Lethal recessive mutations can significantly increase male (haploid) mortality and their effect should be estimated to provide the appropriate null hypothesis with which to compare observed sex ratios before considering any more complex, sociobiological hypothesis. Prospective maternal care⁴ and local mate competition^{1,2} will also affect sex ratio, but increased haploid mortality due to mutation rate is the only effect common to all haplodiploid species. Using emergence data for some parasitoid species, the rate of mutation to lethal recessives is estimated to be about 0.035 per genome per generation giving a differential haploid mortality of 10%.

The selective forces affecting sex ratio were first considered in detail by Fisher⁹ who concluded that an equal investment by a parent in male and female offspring would be evolutionarily stable in a randomly mating population. In arrhenotokous (haplodiploid) species, the primary sex ratio is determined by mated females laying both fertilized (female) and unfertilized (male) eggs. Biased adult sex ratios are well known in groups such as the Hymenoptera and the Acarina; possible evolutionary explanations have included local mate competition^{1,2}, inbreeding with stochastic effects³, and a variety of approaches based on asymmetrical relationships^{4–8}.

All of these explanations ignore a fundamental genetical feature of arrhenotoky which must always contribute to differential survival and hence to a biased sex ratio. A haploid male cannot survive if he carries a lethal recessive mutation and his viability will be severely reduced by non-lethal but deleterious recessive alleles. Mutation seems to have been ignored because the mutation rate per locus per generation is very small, but the mutation rate per genome per generation (m) is vastly greater. For instance, although there are difficulties in estimating m because of large amounts of redundant DNA, it is estimated that the total number of gene loci in man is about 3×10^4 with a mutation rate per locus of 10^{-5} , giving a rate per genome of around 0.3 per generation¹⁰. Data from consanguineous marriages give a comparable estimate of the mutation rate to lethals and lethal equivalents of 0.06–0.15 per gamete per generation¹¹. An estimate of m which may be more relevant to haplodiploid arthropods than data from diploid vertebrates has been obtained for sex-linked lethals in *Drosophila melanogaster*¹². The mutation rate for recessive lethals is approximately 0.006 per X chromosome per generation or

about 0.030 per haploid genome per generation, and the estimate of m is increased to 0.058 lethal equivalents per haploid genome per generation when deleterious recessives are taken into account. In the following discussion, m will be treated as though it refers entirely to lethal recessives.

If mutations occur randomly and independently of one another, the distribution per gamete will be Poisson and the probability that a haploid genome receives no new mutations will be $\exp(-m)$; thus the probability that a haploid male dies as a result of one or more new mutations is $1 - \exp(-m)$. An unfertilized egg might also receive with probability $1/2$ a lethal recessive present in the heterozygous state in the mother's genome. The probability that a female will not have received any new lethal mutation via either the maternal or the paternal gamete is $\exp(-2m)$. Summing over all previous maternal generations, the probability that a male receives one or more lethal mutations heterozygous in his mother's genome is:

$$(1 - \exp(-2m))(\frac{1}{2} + \frac{1}{4} + \frac{1}{8} \dots) = 1 - \exp(-2m)$$

Thus, ignoring dominant lethal mutations which are assumed to be very rare, the probability that a male dies because of either a new mutation or the mutational load in the female genome is:

$$\begin{aligned} P &= (1 - \exp(-m)) + (1 - \exp(-2m)) \\ &\quad - (1 - \exp(-m))(1 - \exp(-2m)) \\ &= 1 - \exp(-3m) \end{aligned} \quad (1)$$

Equation (1) can be derived from more than one approach and represents a special case of the balance of selection and mutation for sex-linked recessives¹³.

Maynard Smith (personal communication) has suggested that an intuitive interpretation of equation (1) is that the parents of a mixed brood suffer $3m$ new mutations per generation, on average, and all must be eliminated in males. Hence the survival rate of haploids relative to diploids is $\exp(-3m)$ simply as a consequence of the mutation rate, and an initially equal number of fertilized and unfertilized eggs will always lead to an average bias towards females in emergent adults. Numbers seem to be the appropriate measure of investment in the absence of maternal care⁵ as there is no reason why fertilized and unfertilized eggs should represent different costs to the mother. Figure 1 shows the expected female:male ratio given an equal initial numerical investment in the sexes plotted against mutation rate per haploid genome m . The female bias obviously increases with m , and the logarithm of the sex ratio is linear in m . Note that the calculations assume that lethal recessive alleles cause no loss of viability in heterozygotes, and that the probability of homozygosity of a lethal allele is negligible (of the order of the mutation rate per locus per generation).

The importance of equation (1) is that it provides the correct null hypothesis against which any sociobiological explanation of a haplodiploid sex ratio should be tested; clearly a 1:1 ratio is not the expected consequence of an initially equal numerical investment. The difficulty which arises is that the appropriate

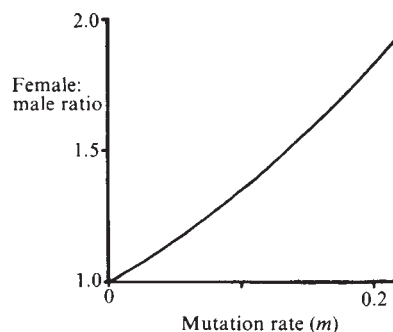


Fig. 1 Expected female:male ratio of adults, given an equal numerical investment in eggs, in relation to mutation rate per haploid genome per generation.

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Table 1 Differential haploid mortality and estimation of mutation rate (m) in gregarious *Apanteles* species

Species		<i>A. chares</i>	<i>A. ruficrus</i>	<i>A. abjectus</i>	<i>A. zygaenarum</i>	Total
All-male broods	Larvae	11	35	11	19	76
	Adults	7	33	8	8	56
Mixed broods	Larvae	6	51	169	335	561
	Adult males	1	4	43	124	172
	Adult females	3	46	103	117	269
Maximum likelihood estimate of haploid:diploid survival:		0.900				
95% confidence interval:		0.838 to 0.957				
Estimated mutation rate per genome per generation:		0.035				
95% confidence interval:		0.015 to 0.059				

Mortality of males and females is estimated using the total emergence data. The mortality considered here relates only to the time between parasite larvae leaving the hosts and emerging as adult parasites from their cocoons. Because mortality in earlier developmental stages has been ignored, m may well have been underestimated. Other mortality factors such as hyperparasitism or starvation have been assumed not to be differential. The maximum likelihood estimate was found by numerical exploration of the likelihood surface. The distribution of twice the log likelihood ratio is chi-squared, allowing the determination of a 95% confidence interval.

null hypothesis cannot be quantified without knowledge of the parameter m in any particular case. An approximate estimate of P can be obtained from the egg to emergent adult mortality in haploid broods of unmated females, but P would be overestimated if there were any mortality factors affecting both haploids and diploids. An unbiased estimate could, in principle, be obtained by cytological examination of eggs in mixed broods, giving a primary sex ratio for comparison with that of emergent adults. In either case it would be necessary to use sufficiently large numbers to obtain a reasonably precise estimate of P because differential mortality of haploids due to mutation is by nature stochastic and sex ratios will be very variable⁸.

There seem to be no published data which allow estimation of P by comparing emergence in all-male and in mixed broods because entomologists generally eliminate data from broods where emergence is not close to 100%. However, one of us (M.R.S.) has collected data over a number of years on the emergence of broods of gregarious parasitoid species of the genus *Apanteles* (Hymenoptera: Braconidae) from hosts collected in the field and reared individually in the laboratory. Four out of 22 species produced large all-male broods as well as mixed broods and the data are given in Table 1, along with the consequent estimate of differential haploid mortality P and the mutation rate m . The 95% confidence interval for P shows clearly that haploids suffered significantly greater mortality than diploids. The maximum likelihood estimate $m = 0.035$ is remarkably close to Mukai's value¹² of 0.030–0.058 for *D. melanogaster*, which suggests that these figures are of the correct order of magnitude for haplodiploid arthropods. The consequent differential mortality of haploids relative to diploids is too large to be ignored and therefore the mutation rate is of some significance when formulating the correct null hypothesis associated with a primary sex ratio of 1:1.

It could be argued that factors other than the mutation rate might cause differential mortality in the two sexes (compare with ref. 14), but in haplodiploid species it is difficult to see how differential pre-emergent mortality in identical rearing conditions could be explained other than by lethal or strongly deleterious recessive alleles. Extreme female-biased ratios which cannot be explained by mutation alone are seen where inbreeding is combined with arrhenotoky¹, and any consequent local mate competition between brothers seems to be reduced by producing often only one or two males in a brood. In these circumstances a haploid male is effectively no more than his mother's gamete and fitness is defined as number of female offspring^{1,3}. A full theoretical treatment of optimum sex ratios in inbred arrhenotokous species should include not only stochastic survival³ but also differential haploid mortality as predicted by equation (1). It is interesting that inbreeding in haplodiploids will not increase female mortality through increasing homozygosity of lethal recessives because these are always eliminated in males. Similarly, because every female has a male parent and every male has a female parent, the increase in homozygosity of

non-lethal but deleterious recessives will affect both sexes equally at equilibrium. Sex ratio arguments based on parental care⁵ are unaffected by the effect discussed here only if differential mutational mortality occurs before the stage at which care is invested. Thus it is clear that mutations can have a significant effect and should therefore be taken into account by sociobiologists considering haplodiploid sex ratios.

We thank John Maynard Smith for noting an error in an earlier version of equation (1), and Roger Mead for advice on exploring messy likelihood functions.

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Eyes and classification of malacostracan crustaceans

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During the past 100 yr various methods of classifying mysids, krill, prawns, shrimps, crabs and lobsters have been proposed, including the use of behaviour, gill type and the relationship of the carapace with the head and the thoracic segments. At least two factors have been ignored in all these schemes—larval characters and optical mechanisms. A survey of malacostracan crustaceans reveals three types of optical mechanism. The simplest uses apposition optics, often with hexagonal facets, and is found in all decapod larvae and most adult brachyuran crabs. Refracting, superposition eyes with hexagonal facets occur in mysids and euphausiids, and reflecting, superposition eyes with square facets using mirror optics are found in adult prawns, shrimps, macrurans such as lobsters, and dromiid 'crabs'. Artificial relationships have been imposed on malacostracan crustaceans by a failure to incorporate eye structure in schemes of classification. Once evolved, eye structure is a stable character and provides evidence for the continued grouping together of penaeid, caridean and stenopid prawns and shrimps; it demonstrates their affinity with lobsters and other Macrura, and confirms the unity of a re-defined Brachyura and the weakness of the Anomura as a coherent taxon.

The classification of decapods has been based on adult characters. Boas¹ and Borradaile² used behaviour, Bate⁴ and Burkenroad³ gills, Laurent⁵ structure of the head and its relationship with the carapace together with the degree of development before eclosion, and Guinot⁶ the position of genital openings. It is difficult to frame diagnoses for larval groups using such frameworks of higher taxa based solely on characters of the adult. Larval characters have not been included in these schemes, yet ideally a natural classification should encompass both adult and larval stages⁷.

Recent work on decapod development and larval adaptation⁸⁻¹¹ and optical mechanism in crustacean eyes¹²⁻¹⁴ has led to a re-examination of decapod relationships. It was assumed that the compound eyes of crustaceans used the same refracting, superposition optical mechanism described by Exner¹⁵ for nocturnal insects. Kuiper¹⁶ pointed out that the peripheral optical elements in crustaceans did not have a sufficiently high refractive index and that they were too uniform to provide the continuous bending necessary. Vogt¹⁷ and Land¹² independently discovered that superposition optics in certain Crustacea were achieved not by lenses but by mirrors and reflection. Grenacher¹⁸ reported that individual facets were square in the compound eyes of prawns and shrimps and certain other groups of Macrura, but did not suggest that this was linked with a special optical mechanism. Square facets are, in fact, essential to the mirror mechanism^{19,20}. Compound eyes in most malacostracans, including all decapod larvae and adult Brachyura, have hexagonal facets and the optical mechanism is one of apposition with no clear zone, a mosaic image being built up from individual ommatidia²¹. Syncarida, Hoplocarida and most Peracarida with well-developed compound eyes such as amphipods and isopods use apposition optics, but loose packing of the surface elements results in circular or irregularly shaped facets rather than closely packed hexagonal facets.

There are at least two exceptions to this usual apposition condition. The first includes mysids and euphausiids, once classified together as the Schizopoda on the basis of biramous limbs retained throughout life. These groups are now placed respectively in the Peracarida (free posterior carapace when

Table 1. Eye structure and classification of selected recent Malacostraca

Taxon	Adult eye type
Subclass Malacostraca	
Series Eumalacostraca	
Superorder Peracarida	
Order Mysidacea	2
Superorder Eucarida	
Order Euphausiacea	2
Order Decapoda	
(Suborder Natantia)	
Section Penaeidea	3
Section Caridea	3
Section Stenopodidea	3
(Suborder Reptantia)	
Section Macrura	
Superfamily Eryonoidea	? (eyes reduced or absent)
Superfamily Scyllaroidea	3
Superfamily Nephropoidea	3
Superfamily Thalassinoidea	1 (eyes poorly developed or reduced)
(Section Anomura)	
Superfamily Galatheoidea	3
Superfamily Dromioidea	3
Superfamily Homoloidea	3 (see text for discussion)
Superfamily Paguroidea	1
Superfamily Hippoidea	1
Section Brachyura	
Subsection Gymnopleura	1
Subsection Oxystomata	1
Subsection Brachygnatha	
Superfamily Brachyrhynchoidea	1
Superfamily Oxyrhynchoidea	1

1, Apposition optics, hexagonal facets; 2, superposition refracting optics, hexagonal facets; 3, superposition reflecting optics, square facets. Taxa of dubious validity are given in parentheses.

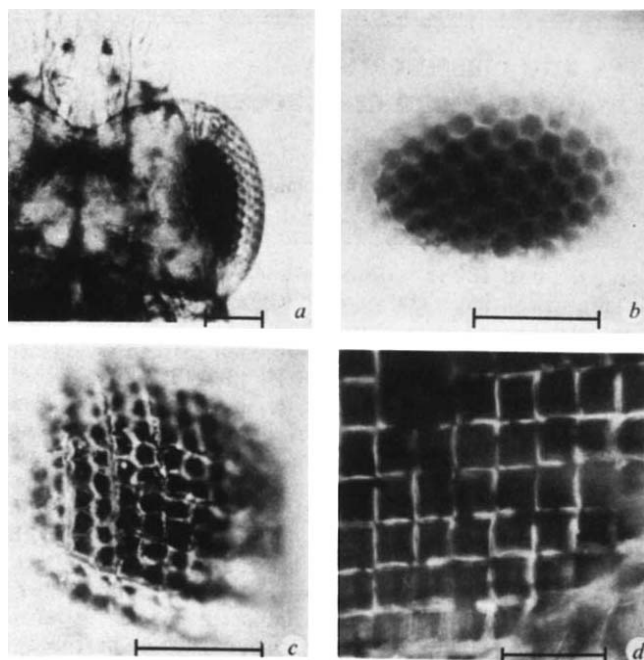


Fig. 1 *Palaemonetes* (*Palaemonetes*) *variens* (Leach, 1814). Stage 1 larva: a, dorsal view of compound apposition eye; b, surface view of hexagonal eye facets. Stage 5 post-larva: c, eye facets 'squaring off'. Adult: d, square facets of superposition reflecting eye. Bar scales represent 0.1 mm. (Photographs: Peter York BM(NH).)

present and oostegites forming a marsupium) and Eucarida (carapace fused to all thoracic segments and often with a long planktonic development). However, Land¹³ reported that mysids and euphausiids both have refracting, superposition eyes in which the essential clear zone is present together with crystalline refractive cones and hexagonal facets. This may be a case simply of convergence by mysids and euphausiids, but optical mechanism needs to be taken into account in future phylogenetic studies of these shrimp-like groups, especially in view of the suggestion²² that mysid-like types may have given rise to euphausiids and to decapods. The presence of a marsupium seems inadequate as evidence for the retention of such disparate orders as amphipods, isopods, cumaceans, tanaids and mysids together in the Peracarida.

The second exception to the usual apposition form of crustacean compound eye is superposition, represented by reflecting eyes with square facets which are found only in adult prawns and shrimps, Macrura and certain Anomura. The larvae of these groups begin their planktonic existence with hexagonal-faceted apposition eyes. There is such a great change in life style and morphology at metamorphosis that this would seem to be the most likely time to convert hexagonal facets to square facets on the surface of the compound eye in those groups which have this form in the adult. However, in the ditch shrimp *Palaemonetes* (*Palaemonetes*) *variens* (Leach, 1814) and other palaemonids⁹⁻¹¹, there is an hexagonal array in all larval stages (Fig. 1a, b) and the facets remain hexagonal until the fifth post-larval moult when the carapace is about 2.8 mm long. There is then a gradual 'squaring off' (Fig. 1c) of the hexagons until the juveniles reach a carapace length of about 5.8 mm. The facets are then perfectly square and arranged in an orthogonal matrix (Fig. 1d). The square-faceted eyes of the adult may still function as apposition eyes in well-lit conditions by migration of

proximal and distal pigment cells across the clear zone (M. F. Land, personal communication).

Not all decapods develop mirror optics. Adult crabs retain hexagonal-faceted apposition eyes, in common with many other groups of crustaceans with compound eyes. An analysis of adult eye type of selected malacostracan groups held in the Crustacea collection at the British Museum (Natural History) is presented in Table 1. Natantia and Macrura with well-developed eyes have at least part of the compound eye composed of square facets. Those burrowing macrurans that have small or poorly developed eyes, such as members of the superfamily Thalassinidea, have hexagonal facets. Section Anomura lies uneasily between the Macrura and Brachyura and contains a wide variety of forms. This is shown clearly by eye structure with square facets in superfamily Galatheaidea and hexagonal facets in superfamilies Paguroidea and Hippoidea. All Brachyura have hexagonal facets with the exception of the Dromioidea in which there are square facets distally with hexagons in the lateral field. This transference of Dromioidea from the Brachyura is strongly supported by larval evidence for the superfamily Dromioidea⁷. However, Rice²³ suggests, again on the evidence of larvae, that the Dromioidea are polyphyletic and that the homolid Dromioidea and raninids of the subsection Gymnopleura are closely related and near to the stem from which the higher Brachyura evolved. Homoloidea would therefore be alone among the Brachyura in having evolved independently square-faceted eyes.

The oldest decapod fossil material²⁴ comes from the Permian-Triassic and includes *Antrimpos* Munster, 1839 of the natant family Penaeidae and *Protoclytiopsis* Birshteyn, 1958 of the astacid family Erymidae (Brachyura are not recorded until the Jurassic). Fossil evidence is equivocal about the structure of eyes as preservation is generally poor, but recent penaeids and lobsters have square-faceted eyes in the adult. Reflecting superposition eyes are found only in prawns, shrimps, long-bodied macrurans and some anomurans. Such an unusual eye type suggests a strong phylogenetic relationship and not simply convergence. As it is likely that this optical mechanism arose early in decapod evolution, eye structure will be useful as an indicator of affinity. This in turn provides a strong case for maintaining penaeid, caridean and stenopid prawns as a group, in contrast to recent proposals⁵ for their division, and also emphasizes their link with the Macrura. Suborder Reptantia becomes an untenable taxon containing a mixture of eye types. Anomura has long been recognized as a taxon of convenience⁷ rather than a natural grouping and its constituent groups should be redistributed (Table 1). On the basis of eye design the Decapoda should be divided into only two groups—Natantia, Macrura, Galatheaidea, Dromioidea and Homoloidea which have the unique reflecting superposition eye mechanism and the true Brachyura in which, together with the Paguroidea and the Hippoidea, the apposition eye present in the larva is retained throughout life.

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Modulation of lateral geniculate neurone excitability by noradrenaline microiontophoresis or locus coeruleus stimulation

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The dorsal lateral geniculate nucleus (LGNd) receives afferents from the brainstem which regulate its capacity to transmit visual information from the retina to the striate cortex¹. One such pathway consists of noradrenaline (NA)-containing fibres originating in the locus coeruleus (LC). These provide a dense, uniform, noradrenergic innervation of the LGNd². Electrical stimulation in the LC region has been reported to enhance the responsiveness of LGNd neurones to afferent excitation³. Although this effect was abolished when brain NA stores were pharmacologically depleted, it was not established as a direct action of NA on LGNd neurones because of the widespread distribution of LC fibres to many parts of the brain and the long latency of the response. Recently, we observed that NA, applied locally by microiontophoresis with low ejection currents, produced a delayed increase in the firing rate of most spontaneously active LGNd neurones, an effect selectively blocked by α -adrenoceptor antagonists⁴. We show here that microiontophoretic NA can mimic the ability of LC stimulation to enhance the synaptic excitation of LGNd neurones. As neither NA nor LC stimulation activated LGNd neurones in the absence of synaptic or glutamate-induced excitation, both appear to act through a neuromodulatory mechanism. The postsynaptic α -adrenoceptor antagonist WB-4101⁵ blocks the facilitation produced by locally applied NA and by coeruleo-geniculate pathway stimulation, providing evidence for pharmacological identity of the two effects.

Extracellular recordings were obtained from single neurones in the LGNd of male albino rats anaesthetized with chloral hydrate. Microiontophoresis was carried out with blunt five-barrel micropipettes to which a fine glass recording pipette was cemented. The electrode assembly was positioned 4.0 mm anterior to lambda and 3.5–4.0 mm lateral to the midline and lowered approximately 4.0 mm below the cortical surface until the LGNd was recognized by cellular responses to movement of a light beam across the visual field. To activate LGNd neurones orthodromically, some animals had a stimulating electrode implanted in the optic chiasm. Further experimental details are given in Fig. 1 legend.

First we examined the effects of iontophoretically applied NA on the response of LGNd neurones to stimulation of the optic chiasm. Suprathreshold shocks usually evoked a single discharge with latency 2–4 ms followed by a period of inhibition typically lasting 70–230 ms. Late activity, consisting of bursts of up to four or five spikes at regular intervals following the inhibitory pause, was exhibited by most cells. This response pattern is characteristic of geniculocortical relay neurones (P-cells)⁶.

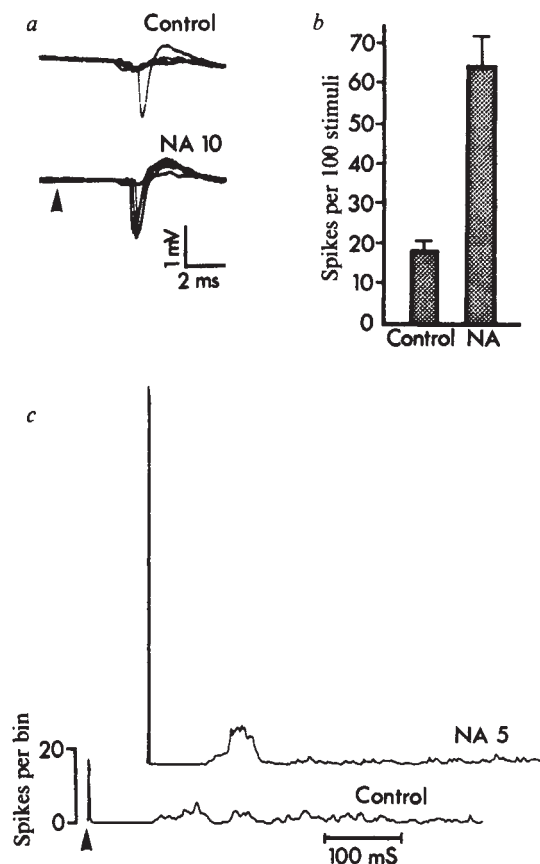


Fig. 1 NA facilitation of unitary action potentials in the LGNd evoked by optic chiasm stimulation. In *a*, subthreshold shocks were applied to the optic chiasm with a concentric bipolar stimulating electrode (SNE-100, Rhodes Medical Instruments) at a frequency of 1 Hz. The stimulus strength was adjusted so that approximately one out of five stimuli produced a short latency spike discharge (upper trace). For the cell shown, 0.5 mA peak to peak, 0.1 ms duration biphasic rectangular pulses satisfied this condition. The position of the stimulus is indicated on the storage oscilloscope record by an arrowhead. Shock artefacts were electronically blanked in these traces. During NA iontophoresis (10 nA, 60 s), four out of five stimuli produce spikes in identical stimulation conditions (lower trace). Five superimposed sweeps. Positivity is upward. *b*, Summary of data from five cells in four animals. The experimental procedure was as in *a* except that each unit was tested with 100 shocks before and during the iontophoresis of NA at 5–20 nA for 2–5 min and this was repeated 2–8 times per cell. Data are the mean $\pm$ s.e.m. of averages for each cell. *c*, Post-stimulus time histogram demonstrating the effect of NA on the early and late responses to optic chiasm stimulation. As well as facilitating the initial spike response (vertical line at left of each histogram), NA (5 nA, 50 s) causes a shift in the late activity to a peak of high probability firing immediately following the inhibitory pause (upper histogram). Histograms were generated with a Nicolet 1072 signal averager. Each histogram represents the summed responses to 100 stimuli delivered at 1 Hz. Stimulation parameters: 1 mA, 0.15 ms. Bin width: 2 ms. In this and subsequent experiments, Sprague-Dawley rats weighing 240–300 g were used. The animals were anaesthetized with chloral hydrate (400 mg kg⁻¹, intraperitoneally); additional anaesthetic injections were given as necessary. Core temperature (monitored with a rectal probe) was maintained above 36°C with a heating pad. Extracellular unitary action potentials were recorded using microelectrode assemblies consisting of a five-barrel micropipette array (15–25 μ m in diameter) to which a fine single-barrel recording electrode (~1 μ m tip diameter) was affixed with dental acrylic. The tip of the recording electrode extended 20–40 μ m beyond the iontophoresis barrels. The recording electrode contained 2% pontamine sky blue in 2 M NaCl and typically had an impedance of 6–12 M Ω . Solutions used for microiontophoresis were: L-noradrenaline D-bitartrate (Regis), 0.1 M, pH 4; serotonin creatinine sulphate (Regis), 0.04 M, pH 4; L-glutamic acid monosodium salt (Sigma), 0.5 M, pH 8; picrotoxin (Sigma), saturated solution in 0.15 M NaCl, pH 4; and WB-4101 (2-([2',6'-dimethoxy]phenoxyethylamino)methylbenzodioxan HCl; WB Pharmaceuticals), 0.1 M, pH 4. A retaining current of -10 nA was applied to the iontophoresis barrels between periods of ejection except when glutamate was used in which case the backing current was +10 nA. To minimize polarization artefacts, a balancing current was continuously passed through an electrode barrel containing 4 M NaCl. Electrode placement was verified by ejecting dye iontophoretically from the recording pipette and identifying the spot thus produced in brain sections prepared by routine histological procedures.

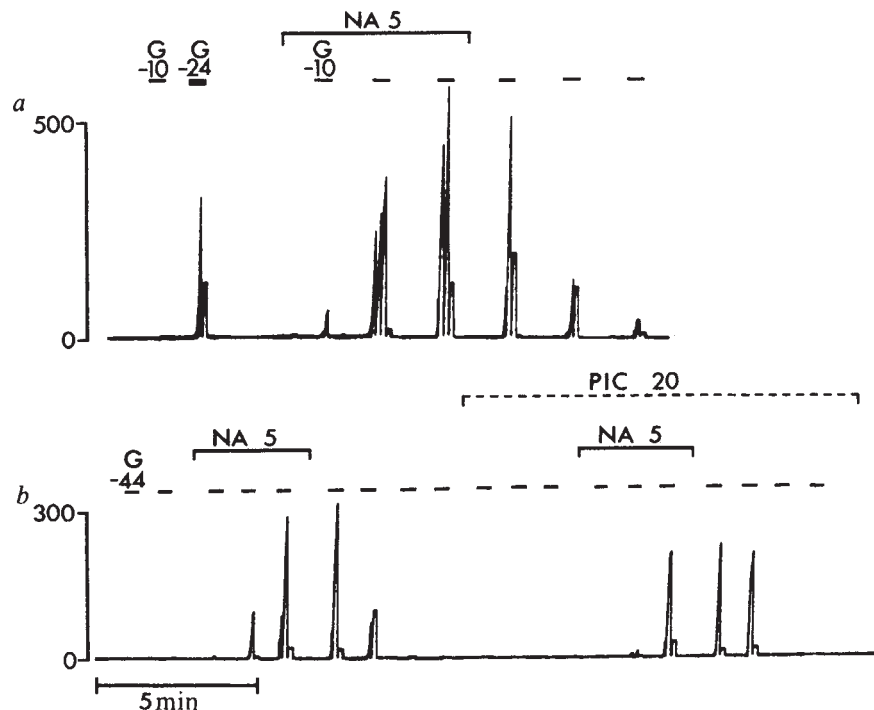
Local application of NA by microiontophoresis caused an increase in the probability of spike generation during the early phase of the response to chiasmatic stimulation (Fig. 1*a–c*). In addition, late spikes tended to occur at earlier times following the stimulus and frequently a peak of high probability late firing was observed immediately after the inhibitory pause (Fig. 1*c*). To obtain these effects, NA was applied with iontophoretic currents of 5–20 nA for periods of 1–5 min. (Low iontophoretic currents were exclusively used because recently⁴ we found that higher currents may produce a loss of responsiveness or have weak depressant effects. This may, in part, account for the variable effects of NA noted by previous investigators^{7–9}.) In some cases the stimulus intensity was adjusted so that approximately 20% of the stimuli produced initial spikes. In these conditions, iontophoresis of NA resulted in a 255% increase in the frequency of spike generation (Fig. 1*a, b*). In contrast, in paired comparisons with NA, serotonin, another monoamine present within axon terminals in the LGNd^{10,11}, always depressed responses to optic chiasm stimulation (5–10 nA; six cells), as has been reported by others^{7,8}.

These results suggested that NA could facilitate the responsiveness of LGNd relay neurones to retinal ganglion cell excitation and ultimately enhance signal transfer from retina to visual cortex. To determine whether this was due to a direct excitatory action of NA or to an interaction of the amine with afferent synaptic activation, ongoing tonic excitation of the relay cells was suppressed by section of the optic nerve behind the globe of each eye^{12,13}. Following this, NA generally failed to excite silent LGNd units although the same cells could be activated easily by iontophoresis of the excitatory amino acid glutamate. Thus NA, in contrast to glutamate, did not seem to be a direct excitant. However, low currents of NA (2–15 nA) were able to facilitate the excitatory action of the amino acid dramatically. Iontophoresis of glutamate at currents which produced little or no excitation before application of NA, resulted, with concurrent ejection of NA, in marked activation of firing (22 cells in 14 rats) (Fig. 2*a*). The effect of NA took 30–90 s to develop and lasted up to 6 min following cessation of the ejection.

The ability of NA to produce enhanced responsiveness to glutamate suggested that NA could facilitate the general excitability of relay neurones, presumably through a postsynaptic mechanism. However, it has been speculated that the facilitatory effects of conditioning LC stimulation are due to suppression of inhibitory interneurons³ and it is possible that locally applied NA acts indirectly as well. Two populations of local inhibitory elements seem to synapse on P-cells: perigeniculate reticular (PGR) neurones located in the thalamic reticular nucleus¹⁴ and intrinsic interneurons (I-cells)¹⁵. Arguments against the participation of PGR neurones in the facilitatory action of iontophoretic NA are: first, NA generally produced only a small decrease in the postexcitation inhibitory period believed to be mediated by PGR cells, yet the responsiveness, especially of the short latency spike, was markedly facilitated. Second, the activity of NA was independent of the proximity of the iontophoretic pipette to the reticular nucleus which is situated rostral to the geniculate, as much as 1.5 mm anterior to the caudal extent of the LGNd¹⁴.

However, it is conceivable that I-cells could mediate the action of iontophoretic NA. Recent evidence suggests that γ -aminobutyric acid (GABA) is the neurotransmitter utilized by these cells¹⁶. Therefore, if the facilitatory action of NA on relay neurones was due to depression of I-cell firing or suppression of GABA release from presynaptic I-cell dendrites¹⁷, blockade of GABA-mediated neurotransmission should produce a similar effect. In preliminary experiments, we found that the GABA antagonist picrotoxin was able to block GABA-induced depression of spontaneously active LGNd neurones when iontophoretically applied with currents of 15–20 nA for 1–5 min. Picrotoxin was then applied to 'silent' neurones in optic nerve-sectioned animals. Even with prolonged administration (up to 25 min), the GABA antagonist (15–50 nA) did not produce a facilitation of the response to glutamate (five cells) (Fig. 2*b*).

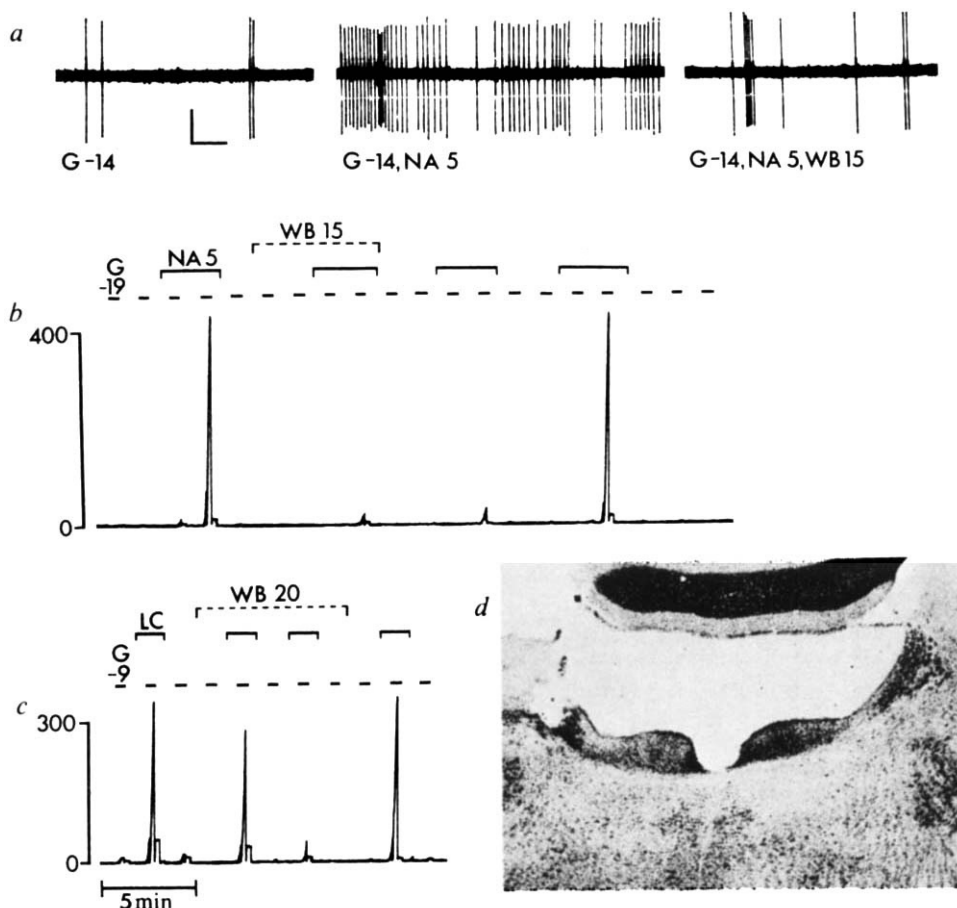
Fig. 2 NA facilitation of glutamate (G)-induced excitation in conditions of suppressed spontaneous activity. *a*, Constant current, 30-s pulses of G were applied to a 'silent' neurone in an acutely optic nerve-sectioned rat. NA causes a reversible potentiation of the response to subthreshold G. In this and subsequent examples, silent neurones were identified by optic chiasm stimulation or iontophoresis of G. Unit activity is expressed as an average rate histogram where the ordinate indicates the number of spikes per 10-s epoch. Duration of iontophoretic ejections are indicated by bars above each record. Numbers refer to iontophoretic currents in nA. *b*, Response of another cell demonstrating that NA but not picrotoxin (PIC) facilitates the action of G (20-s pulses). Note that during the PIC ejection the response to NA is slightly diminished rather than enhanced as would be predicted if the cell were under tonic GABA inhibition.



These observations suggest that the facilitatory action of NA is not mediated transynaptically via adjacent interneurons, although suppression of either class of inhibitory neurone could play a role in the overall physiological action of the coeruleo-geniculate pathway.

To confirm that activity in the coeruleo-geniculate pathway can produce similar effects on cellular excitability as iontophoretically applied NA, we examined the response of LGNd neurones to electrical stimulation in the region of the LC. Concentric bipolar stimulating electrodes were implanted in or

Fig. 3 Blockade by WB-4101 (WB) of the facilitatory actions of NA (*a*, *b*) and LC stimulation (*c*). *a*, Storage oscilloscope tracings of the extracellular spike records of a 'silent' unit in an acutely optic nerve-sectioned rat. The cell was activated by glutamate (G) (left panel) and this response was enhanced by NA (middle panel). WB applied for 16 min antagonized the effect of NA (right panel). Note that the spike amplitude is unaltered during prolonged application of WB. *b*, Rate histogram demonstrating the effect of WB on another cell. *c*, Blockade by WB of LC stimulation-induced facilitation of G in a third cell. LC stimulation does not activate the cell in the absence of G. Stimulation parameters: 0.5 mA, 1 ms, 10 Hz (NE-100 electrode, Rhodes Medical Instruments). *d*, Electrolytic lesion produced at tip of stimulating electrode (20 μ A for 20 s) indicating typical placement in LC. Cresyl violet stained, 50- μ m thick coronal section of formalin-fixed tissue. Position of the electrode tract is marked with an arrow. The arrowhead points to the contralateral LC. V, IVth ventricle. Calibration bar, 1 mm.



alongside the ipsilateral LC (Fig. 3d). Spontaneously active cells in normal animals invariably responded to trains of stimuli applied to the LC (1 ms duration, 10 Hz, 0.3–0.8 mA) with a dramatic increase in firing rate. As with NA iontophoresis, the effect of LC stimulation was delayed in onset (requiring 1–30 s to attain a peak response) and sustained beyond the duration of the stimulus (up to 20 s). In contrast to the effects on spontaneously active cells, 'silent' cells in optic nerve-sectioned animals were not activated directly by LC stimulation. However, as with iontophoretic NA, LC stimulation markedly enhanced the excitatory action of glutamate (five cells) (Fig. 3c).

For further evidence that LC stimulation and iontophoretic NA evoke identical postsynaptic actions, we examined whether the enhanced excitability each produced was sensitive to the α -adrenoceptor antagonist WB-4101. In four cells, WB-4101 at currents of 15–30 nA produced a blockade of the facilitatory action of NA (5 nA) (Fig. 3b). Spike amplitude was unaffected by WB-4101 (Fig. 3a) suggesting that local anaesthetic effects do not occur at the doses used. Similar iontophoretic currents also markedly attenuated the facilitatory action of LC stimulation (three cells) (Fig. 3c). As both the responses to LC stimulation and iontophoretic NA are antagonized by WB-4101, we conclude that the effects of stimulation of the LC are mediated by NA released from the terminals of noradrenergic fibres located in the vicinity of LGNd neurones.

These results support the concept that brain noradrenergic neurones act primarily to modify the responsiveness of their target cells to convergent inputs carried by other afferent channels¹⁸. This phenomenon has been termed 'neuromodulation' to distinguish it from the action of the conventional neurotransmitters which produce direct excitation or inhibition^{19,20}. Modulatory transmitters typically have little effect on cellular activity in themselves and the changes in excitability they produce are usually slow to develop and prolonged in duration. The properties of NA action in the LGNd are those of neuromodulation and exemplify a common neuromodulatory role of monoaminergic neurotransmitters in invertebrate^{21–25} and vertebrate^{18,26–30} nervous systems. However, an understanding of the detailed cellular mechanisms mediating the facilitatory actions of NA on LGNd neurones requires analysis with intracellular recording techniques.

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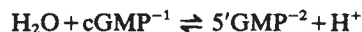
ATP mediates rapid reversal of cyclic GMP phosphodiesterase activation in visual receptor membranes

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Weak or strong lights will activate visual receptor rod disk membrane (RDM) cyclic GMP phosphodiesterase (PDE) in the presence of GTP cofactor^{1,2}. A similarly activated GTPase can exhaust small amounts of initially present GTP to deactivate the PDE. However, further additions of GTP reactivate PDE without more light, and deactivation by simple GTP depletion takes minutes or more, even at GTP concentrations 100 to 1,000 times lower than physiological levels^{1,2}. A more rapid deactivation mechanism must exist if modulation of cytoplasmic cyclic GMP by light is to play a role on the time scale (seconds) of events in vision^{3,4}. We report here that ATP is essential to such rapid control and that its presence permits multiple cycles of activation–deactivation. The complete control mechanism seems to involve gamma phosphate transfer from both ATP and GTP.

Bovine RDM suspensions were prepared from fresh or frozen retinas in IR light using standard procedures⁵. Cyclic GMP PDE activity was measured continuously using a fast recording pH meter to assay the stoichiometric production of protons⁶, in the reaction



A xenon photoflash equipped with calibrated attenuator filters was used to activate PDE. Light titration of the hydrolytic velocity with GTP cofactor showed saturation kinetics with half-maximal activation at bleaches of 1 part in 10,000–20,000. For all experiments reported here, the activating bleach was kept at 1 part per 50,000, within the linear range of the hydrolytic velocity response to the bleaching stimulus³.

Figure 1, curve *a*, shows the formation of protons associated with cyclic GMP hydrolysis on flash activation in the presence of 60 μM GTP. Given sufficient substrate, cyclic GMP, the hydrolysis seen in curve *a* continues for more than 2 min while curves *b* and *c* show that a pulse of 8 μM ATP can arrest or 'quench' the activity within a few seconds anytime after the light activation. If this quenching effect were due to ATP competitive inhibition at the GTP binding site, one might expect ATP to be even more effective if added before the flash. This is not the case as seen in curve *d*. Activation takes place just as in the previous curves and is quenched with the same delay. Curve *e* shows that 8 μM ATP alone cannot support activation but that quenching has still occurred, as subsequent addition of GTP at times exceeding the characteristic quench delay shown in *b*, *c* and *d* can no longer produce activation. Yet after the GTP is available, a flash again produces a normal activation–quench cycle. Curve *f* and its derivative, *g*, show that this cycle can be repeated indefinitely.

In previous work, it was shown that the GTP analogues, GMP-PNP and GMP-PCP, are efficient cofactors of activation^{1,2}. However, Fig. 2A shows that activation with GMP-PNP is not efficiently quenched by ATP (compare curves *a* and *e*). Nor is it possible to quench GTP-mediated activation using the ATP analogue, AMP-PNP (curve *b*). Instead, AMP-PNP competitively blocks the action of ATP (curve *d*).

Figure 2B shows that the efficiency of quench increases with ATP concentration. We find (Fig. 2C) that GTP alone can quench PDE activity, though both required concentration and maximum effectiveness show it to be a poor substitute for ATP. The quench process has a K_m of about 4 μM for ATP and about

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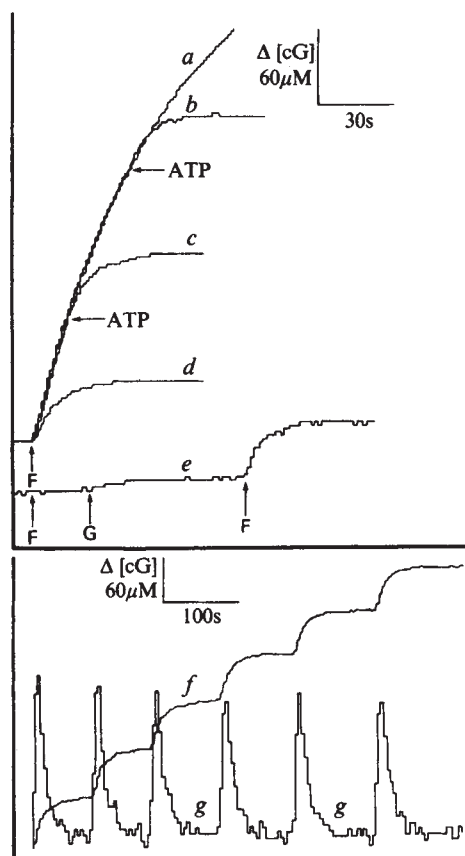
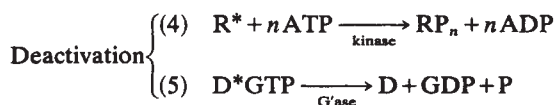
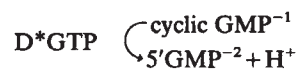
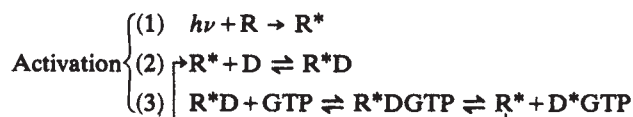


Fig. 1 Stoichiometric proton release with cyclic GMP hydrolysis following a flash, F, of 1-ms duration that bleaches 2.4×10^{-5} rhodopsin in presence of $60 \mu\text{M}$ GTP cofactor (a–d). Curves b–d, $8 \mu\text{M}$ ATP was injected at 25 s, 15 s or –5 s. Curve e, no GTP present until G, 23 s after flash, but ATP present at –5 s before flash. Curve f, cyclic GMP hydrolytic response to series of flashes in presence of 1 mM GTP/ 3 mM Mg-ATP. Curve g, derivative of trace f showing rise and fall of light-activated enzyme velocity. All traces from separate disk membrane samples containing $5 \mu\text{M}$ rhodopsin, 4 mM cyclic GMP, 2 mM MgCl_2 , 150 mM KCl, 20 mM HEPES, 1 mM dithiothreitol, pH 7.9, $T = 32.4^\circ\text{C}$. Buffer limited pH change to 0.1 unit per 1 mmol H^+ added. $100 \mu\text{M}$ HCl or cyclic GMP gave equal deflections after hydrolysis of the latter in activated preparations. Membranes were from frozen retinas for this and subsequent figures. Fresh material gave nearly identical results.

$1,400 \mu\text{M}$ for GTP. In addition, the V_{max} ratio of ATP to GTP in this process is about 2. Thus, the specificity V_{max}/K_m for quenching is about 700 times greater for ATP than for GTP.

It has been shown previously that rhodopsin is phosphorylated on bleaching in the presence of a membrane-bound kinase and ATP^{7,8}. The K_m and specificity of this kinase for both ATP and GTP are close to those of the quenching process reported here⁹. No other protein is known to be phosphorylated on RDMs. We propose that the quenching process reported here, which terminates the potency of bleached rhodopsin to activate PDE, is opsin phosphorylation. A function for opsin phosphorylation has not been previously established.

The results of this and earlier work^{2,3} suggest that light initiates a cycle of activation and deactivation of PDE according to the following scheme:



R^* is the long-lived activator formed by the bleaching of rhodopsin, R. It can be quickly made inactive by phosphorylation [reaction (4)] as suggested by this report. While active, it can bind an inactive PDE molecule, D, and catalyse the binding of GTP to it or to a separate GTP-binding protein which in turn activates D by complexing with it to yield D^*GTP (ref. 10). The active diesterase, D^*GTP , then hydrolyses cyclic GMP until it also is deactivated by action of a GTPase that is specific for the bound form of GTP.

In this scheme, it is apparent why GTPase alone is not capable of deactivating D^* , for the trigger molecule, R^* , is still present and able to mediate activation again and again until it is deactivated by the kinase reaction. The gamma-phosphate lability of both GTP and ATP is required for fully reversible PDE activity: AMP-PNP does not permit R^* to be phosphorylated and GMP-PNP does not permit GTPase to release the bound cofactor to terminate D^* activity. After the activator, R^* , is quenched, a GTPase specific for the bound GTP of the complex D^*GTP

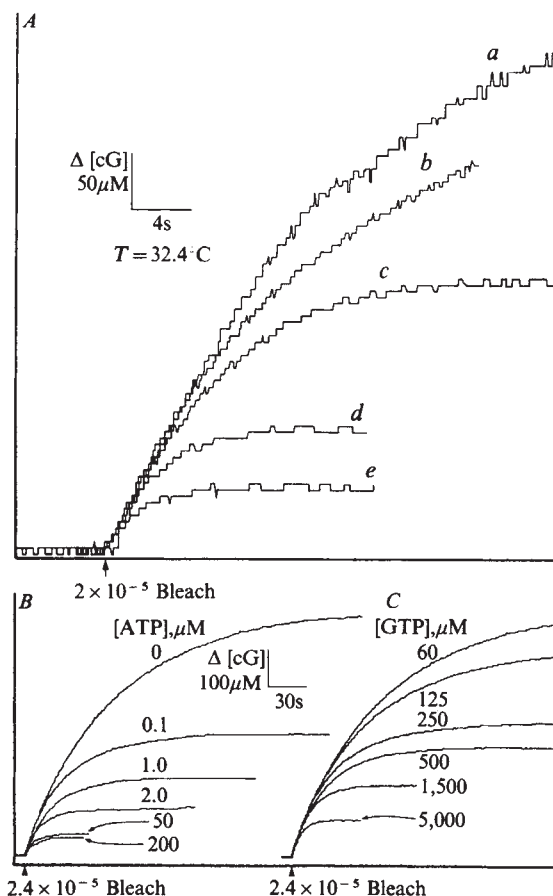


Fig. 2 A, Effect of ATP and GTP analogues on quench. Curve a, $500 \mu\text{M}$ GMP-PNP + $5 \mu\text{M}$ ATP; curve b, $50 \mu\text{M}$ GTP + $100 \mu\text{M}$ AMP-PNP; curve c, $50 \mu\text{M}$ GTP; curve d, $50 \mu\text{M}$ GTP + $5 \mu\text{M}$ ATP + $100 \mu\text{M}$ AMP-PNP; curve e, $50 \mu\text{M}$ GTP + $5 \mu\text{M}$ ATP. B, ATP titration of quench effect. The parameter labelling the curve is the concentration of ATP, in μM . $60 \mu\text{M}$ GTP present for all curves and ATP added 2 s before flash. C, GTP titration of quench effect (no ATP present). Flash F, as described in Fig. 1 legend.

could terminate activity even in the presence of large amounts of ambient but unbound GTP. Thus, the deactivator role of GTPase is not mediated via the cytoplasmic GTP depletion implied by previous work^{1,2}.

It is worthwhile to compare the mechanism proposed here with that of hormonally activated adenylate cyclase, also known to require GTP cofactor¹¹. Both systems activate effectively in GMP-PNP¹² and neither can deactivate without the action of a membrane-bound GTPase¹³. As in RDM, however, it should not be possible for GTPase to deactivate the cyclase mechanism alone. The hormone-receptor complex needs to be deactivated as well. Reduction of blood hormone concentration alone might in some cases lead to adequate dissociation of the hormone-receptor complex to complete the deactivation on a time scale appropriate to needs for control. However, to achieve more rapid regulation, a hormone-receptor might be quenched by phosphorylation in a process similar to that which quenches the capacity of rhodopsin to activate RDM phosphodiesterase.

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Localization of metabolites in animals using ³¹P topical magnetic resonance

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High-resolution phosphorous (³¹P)-NMR spectra of biological molecules provide detailed information about the metabolism of living systems¹⁻⁴. Although the NMR method is non-destructive, all studies so far, with two exceptions^{5,6}, have been carried out on excised, perfused organs and tissues or have required some form of surgery² for *in situ* measurements. The use of 'surface' radiofrequency coils⁵ does not require surgery but is best suited for tissues close to the surface of the animals. We describe here 'topical magnetic resonance'—a new, non-surgical method for acquiring ³¹P-NMR spectra from a selected, localized place deep within an animal by modifying the main magnetic field, B_0 , using only static-field gradients. The method is conceptually similar to one spin-imaging method⁷ but primarily provides biochemical rather than spatial information. This new technique can be used in fundamental investigations into living systems, clinical diagnosis and the estimation of the efficacy of drug therapy.

To limit the volume from which the high-resolution ³¹P-NMR signals are received, the effective homogeneous volume of B_0 must be reduced and centred on the region of interest. The topical magnetic resonance (TMR) technique consists of superimposing high-order, static magnetic field gradients on to B_0 via room-temperature magnetic field profiling coils mounted coaxially inside the bore of the magnet.

The magnetic field, $\beta(r, \theta)$, generated by a profile-coil system with azimuthal symmetry, can be described in terms of a Taylor expansion about the origin⁸,

$$\beta(r, \theta) = \sum_{n=0}^{\infty} \beta_n r^n P_n(\cos \theta) \quad (1)$$

where $\beta(r, \theta)$ is the magnetic field at (r, θ) , $P_n(\cos \theta)$ are the Legendre polynomials of order n , and the field derivatives, β_n , are defined by the coil geometry and the d.c. current flowing through the coil system. A sensitive volume of homogeneous field can be delineated, centred on the origin and surrounded by inhomogeneous field gradients. A detailed discussion of the rationale of coil design will be given elsewhere.

The description of the magnetic field profile is simplified if only fields along the z -axis ($r = z, \theta = 0$) are considered. In a coil system of maximum order $n = 4$ where $\beta_0 = 0$ and β_4 is negative, the resultant profile along the z -axis produced by the sum of B_0 and $\beta(z)$ is shown in Fig. 1.

The inhomogeneity, ΔB , across the central region of axial extent, $2a$, is given by:

$$\Delta B = \frac{\beta_2^2}{2\beta_4} \quad (2)$$

where

$$2a = 3.1 \left(\frac{\beta_2}{\beta_4} \right)^{1/2} \quad (3)$$

By varying β_2 and β_4 the sensitive volume can be adjusted to match that of the centrally placed region within any given object.

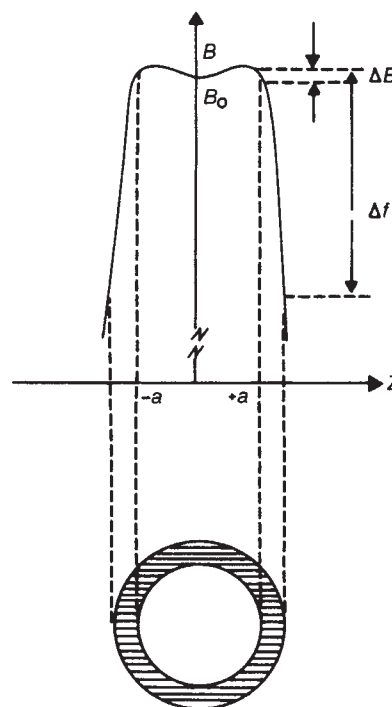


Fig. 1 The profile of the magnetic field along the z -axis in TMR. The inhomogeneity, ΔB , within the central region shown schematically in the projection below, is constrained to remain less than the typical ³¹P linewidths. In the shaded region the ³¹P lines will be broadened by the field gradients.

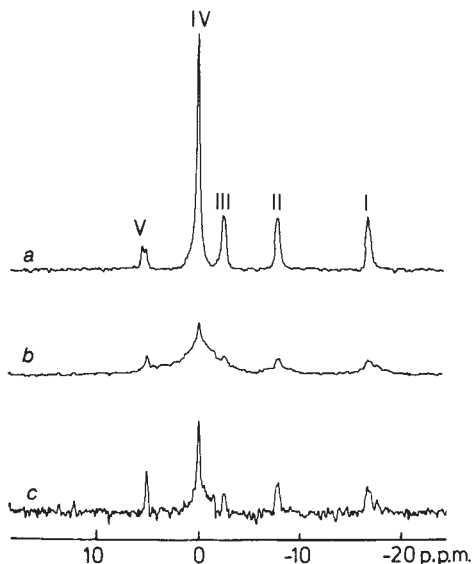


Fig. 2 ^{31}P -NMR spectrum of a two-compartment phantom containing ATP (signals I, II and III), phosphocreatine (IV) and inorganic phosphate (V). A single-turn saddle-shaped radio-frequency coil operating at 73.8 MHz was used in a standard wide-bore (89 mm) superconducting magnet. Profiling was obtained with a coil system designed to provide spatial localization across an internal volume ($2a \sim 15\text{--}45$ mm) whilst maintaining a homogeneity of ~ 0.1 p.p.m. at an operating B_0 field of 4.3T. The working bore of the system was 45 mm. The spherical compartment contained 4.65 mM ATP, 9.30 mM PCr and 3.72 mM P_i , and the cylindrical compartment contained 4.65 mM ATP, 18.60 mM PCr and 1.86 mM P_i . *a*, Spectrum (300 scans) in the absence of localizing fields. A 6-Hz line broadening exponential multiplication was used to enhance the signal-to-noise ratio. *b*, Spectrum (600 scans) with the axial extent reduced to 20 mm. The same line broadening was used as in *a*. *c*, Spectrum obtained on removing the broad component of spectrum *b* by convolution difference⁹. Line broadenings of 6 and 60 Hz were used and the vertical scale in the spectrum was multiplied by 4 for clarity of presentation. Chemical shifts are defined as positive in the high-frequency direction with phosphocreatine taken as an internal reference.

Adjacent to the sensitive volume lie regions where the magnetic field changes vary rapidly with position, causing broadening of the spectral lines. The radiofrequency coil will receive signals from the whole sample so the total signal will contain both the narrow, high-resolution lines and the inhomogeneously broadened lines which must be separated out to recover the high-resolution spectrum of the sensitive volume.

Experiments were carried out on a two-compartment test phantom consisting of a cylindrical compartment of diameter 30 mm surrounding a spherical compartment of diameter 20 mm, each containing ATP, phosphocreatine (PCr) and inorganic phosphate (P_i) (for concentrations, see Fig. 2 legend), dissolved in 150 mM KCl to simulate the electrical conductivity of tissue. The internal pH was adjusted to be 0.35 units less than that of the external pH, thereby producing a chemical shift difference between the P_i resonant frequencies of the two samples.

The spectrum of the sample was obtained with the axial extent set to 45 mm (Fig. 2*a*). The three ATP peaks and the PCr peak are unaffected by the difference in pH, whereas two clearly distinguishable P_i peaks appear. Using the PCr peak as a reference, the internal and external P_i peaks correspond to pH values of 7.10 and 7.45 respectively. The P_i concentration ratio measured from the peak areas is in good agreement with the expected value.

When the axial extent is reduced to 20 mm, a broad component at the base of each peak arises from the metabolites in the external compartment, which now lie in a region of

inhomogeneous magnetic field (Fig. 2*b*). This broad signal is eliminated using the convolution difference technique⁹ (Fig. 2*c*). The narrow peaks correspond to the metabolites present only in the spherical compartment, and the pH of the P_i peak in this spectrum is 7.10, in agreement with the position measured previously.

To demonstrate the potential of the method in animal experiments, we have studied the liver of a live rat. In the spectrum obtained from the rat in the absence of any localizing fields, the ATP and PCr peaks are readily identifiable, but the P_i and the sugar-phosphate region of the spectrum is less readily assigned because the 2,3-diphosphoglycerate from blood contributes two signals at ~ 5.0 and 6.5 p.p.m. and the first of these overlaps with the P_i signal (Fig. 3*a*). Blood contributes a negligible amount to the ATP peaks. Therefore the ATP signals arise primarily from liver and muscle tissue, the PCr arises exclusively from muscle, whereas the signals in the P_i and sugar-phosphate region may have contributions from liver, muscle and blood.

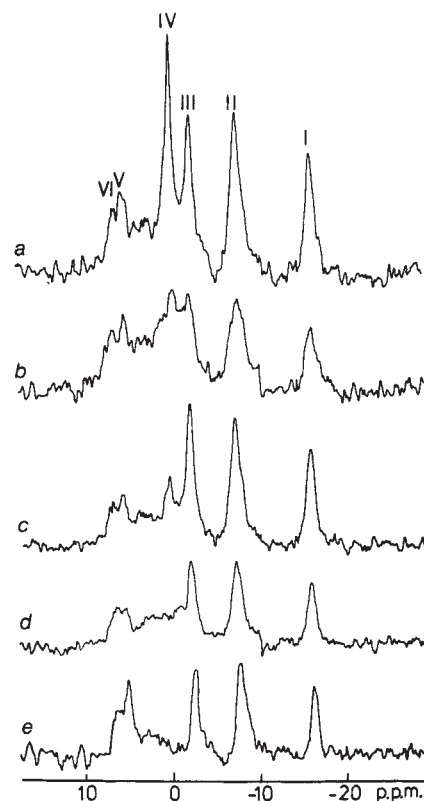


Fig. 3 Spectra *a*–*d* were obtained from an intact rat anaesthetized with pentobarbitol. 150-g male Wistar rats, starved for 24 h, were used for all experiments. Signal assignments are: I, II and III, the β -, α - and γ -phosphate, respectively, of ATP; IV, phosphocreatine; V, inorganic phosphate; VI, sugar phosphate, AMP and IMP. Peaks V and VI may also contain contributions from 2,3-diphosphoglycerate in the blood. Peak II contains a small contribution from ADP and peak III has contributions from ADP, NAD and NADH. *a*, Spectrum in the absence of localizing fields ($128 \times 90^\circ$ pulses at 2-s intervals). *b*, The same as *a* with localization (axial extent 20 mm). *c*, Spectrum in the absence of localizing fields ($1,024 \times 90^\circ$ pulses at 220-ms intervals). *d*, The same as *c* with localization (axial extent 20 mm). *e*, Spectrum of a perfused rat liver ($480 \times 90^\circ$ pulses at 220-ms intervals). For clarity of presentation the spectra in *c* and *d* were divided by 4. The broad components were eliminated using the convolution difference technique⁹ with line broadenings of 16 and 233 Hz. Chemical shifts are defined as positive in the high-frequency direction, and phosphocreatine is taken as an internal reference. Measurement of T_1 , using progressive saturation technique¹¹, on the β -phosphate resonance of ATP gave a value of 0.14 s in the presence of localizing fields. This is characteristic of ATP in liver. The remaining small PCr signal in *b* is likely to arise from the diaphragm.

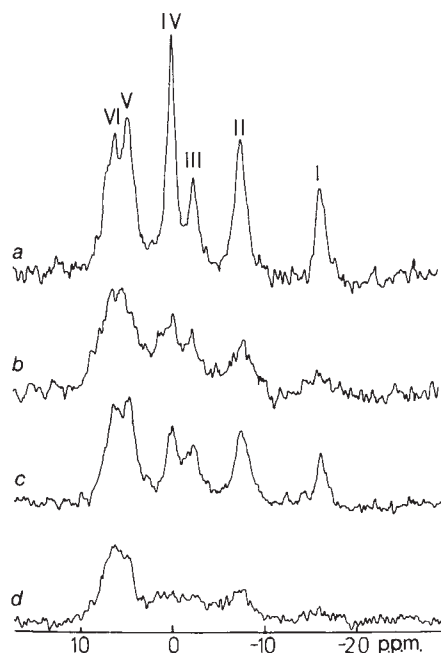


Fig. 4 ^{31}P -NMR spectra of a rat after surgery to cut off the hepatic blood supply. The spectrometer conditions were the same as in Fig. 3 legend. Spectra a–d are analogous to spectra a–d of Fig. 3.

manner. The method is easily extended for the investigation of other organs of laboratory animals.

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Excretion, isolation and structure of a new phenolic constituent of female urine

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The regular occurrence of a peak due to an unidentified substance (X) in the gas chromatographic traces obtained from phenolic extracts of urine from human pregnant and non-pregnant females has been reported¹. The biphasic excretion of X with maxima in the luteal phase of the ovulatory cycle and relatively high levels in the first trimester of pregnancy were noteworthy and suggested that the substance may have a biological significance. Close similarities between the excretory pattern, the chemical and chromatographic properties of X and of those of the known phenolic steroids suggested initially that this compound was steroidal in nature. The same, or a similar, substance seems to be excreted in the vervet monkey (*Cercopithecus aethiops pygerythrus*)². We now report the excretory pattern of X in more detail, the isolation of the pure compound from pooled pregnancy urine and the chemical structure. The structure determined by mass spectrometry, IR spectroscopy and NMR spectrometry is: *trans*-(±)-3,4-bis[(3-hydroxyphenyl)methyl]dihydro-2-(3H)-furanone (HPMF) and was confirmed by synthesis.

Sequential analyses were carried out on urine collected daily: (1) by volunteers throughout the normal ovulatory cycle; (2) when monitoring oestrogen excretion in patients who were being treated for anovulatory infertility following treatment with postmenopausal gonadotropin; and (3) when following the urinary excretion of oestrogens and of X from conception to term³. After acid hydrolysis of the urinary steroid and other conjugates, phenolic fractions were prepared, the extracts acetylated and analysed by gas liquid chromatography (GLC) using a Pye 104 gas chromatographic system³. Figure 1 shows

The sensitive volume was reduced ($2a = 20$ mm) and the resulting TMR spectrum is shown in Fig. 3b. The smaller peak intensities indicate that now high-resolution signal is being acquired from a smaller volume, and as the spectra of Fig. 3a and b were obtained with the same spectrometer conditions, there will be an inevitable reduction in signal-to-noise ratio. Nevertheless, a change in the relative proportions of the metabolite resonances is apparent.

The spectra of Fig. 3a and b were obtained using a radio-frequency pulse interval of 2 s, which is typical for ^{31}P -NMR studies of whole tissue. Figure 3c shows a spectrum obtained with a pulse interval of 220 ms, in the absence of localizing fields. The PCr peak intensity is now significantly reduced in comparison with its intensity in Fig. 3a, because its spin-lattice relaxation time, T_1 , is long (~ 3 s). The reduction in the ATP peak intensities is far less, as the ATP in tissues such as muscle has a rather shorter relaxation time (~ 1 –2 s), and also because a significant percentage of the ATP is in the liver, and liver ATP has very short T_1 values (~ 100 ms)¹⁰. Figure 3d shows the spectrum obtained using the short pulse interval with the reduced sensitive volume ($2a = 20$ mm). This spectrum contains no PCr signal and is very similar to the spectrum of perfused liver shown in Fig. 3e, which was obtained using the same pulse interval of 220 ms. This suggests that the signals of Fig. 3d are predominantly from the liver. The improvement shown in Fig. 3d can only be obtained using the localizing fields.

The P_i/ATP ratio is lower in the liver of the whole animal (Fig. 3d) than in the perfused liver (Fig. 3e). Previous NMR studies of the brain and skeletal muscle of whole rats also show remarkably low P_i levels⁶.

By cutting off the hepatic blood supply of the animal using surgery, similar measurements were made on the same animal (Fig. 4). The marked reduction of ATP and increased levels of P_i after ligation (Fig. 4b and d) indicate unhealthy ischaemic liver, verifying the origin and interpretation of the previous TMR spectra. In contrast, the PCr signal intensity in Fig. 4a is very similar to that in Fig. 3a indicating that the metabolic state of the muscle is little affected by the operation.

Thus, we have identified *in vivo* liver tissue by the ^{31}P spectral characteristics and the relaxation time of the β -ATP resonance. Global liver ischaemia has been diagnosed in a non-invasive

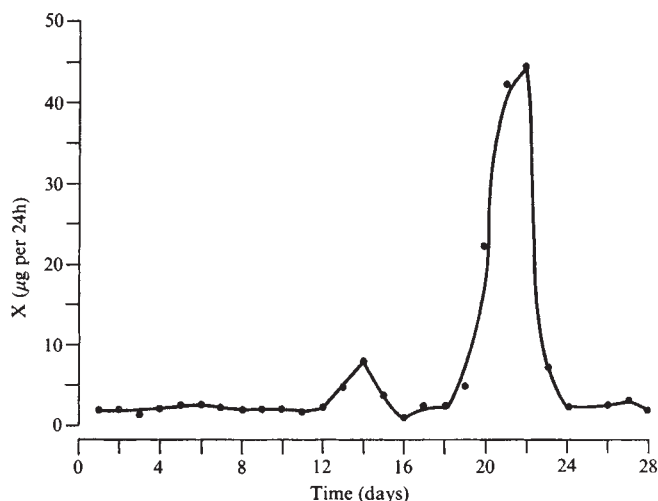


Fig. 1 Cyclic excretion of HPMF in a volunteer with normal ovulatory cycles. 24-h urine specimens were collected daily throughout the cycle and HPMF was extracted from each specimen as described in the text. The phenolic fraction was acetylated and subjected to GLC³ on a Pye 104 gas chromatograph using a 1-m glass column packed with OVI on Gas Chrom Q Support (100–200 mesh). The oven temperature was maintained at 230 °C and the nitrogen carrier gas flow rate was 40 ml min⁻¹. Values obtained are uncorrected for losses which occurred during the chemical extraction and gas chromatography (~40%).

the biphasic excretion of X in a normal ovulatory cycle, maximum excretion being apparent in the luteal phase. In early pregnancy (Fig. 2) excretion of X exceeds that of oestrone, oestradiol or oestriol, but as pregnancy proceeds to term both the absolute excretion of X and the excretion of X relative to the steroidal oestrogens declines.

A purified sample of compound X (10 mg) was isolated for structural analysis from pooled urine (100 l) collected during the first trimester of normal pregnancies. The urine was processed in 2-l batches by passing through a column of XAD2 resin (BDH chemicals) and eluting first with distilled water and then with ethanol to give a fraction containing conjugates of the steroids

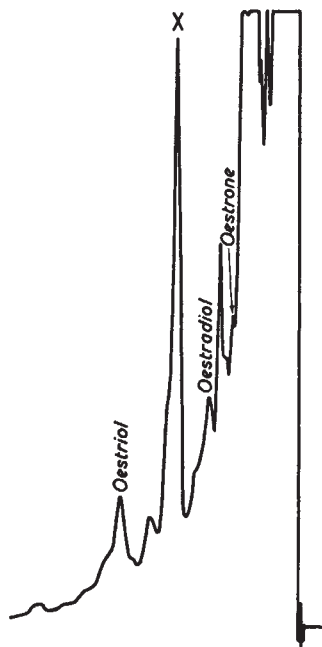


Fig. 2 HPMF was extracted from a 24-h urine specimen, obtained from a patient in the ninth week of pregnancy, as described in the text. The purified phenolic fraction was acetylated and subjected to GLC in the conditions described in Fig. 1 legend.

and of compound X. The ethanol solution was evaporated to dryness under reduced pressure in a rotary evaporator, and the residue was heated on a steam bath for 1 h with aqueous hydrochloric acid (pH 1) to hydrolyse the conjugates. Saturated ammonium chloride was added, and the hydrolysed products were extracted into ether and washed with 8% sodium bicarbonate solution. The phenolic constituents were then extracted with 1 M NaOH solution which was acidified immediately with sulphuric acid. The crude phenolic product was extracted with diethyl ether and purified by chromatography on a column of Sephadex LH20 resin (Pharmacia) and elution with toluene:methanol (9:1, v/v). The fraction containing compound X was purified further by thin-layer chromatography on Kieselgel G (type 60, Merck) for 1.5 h with ethyl acetate:cyclohexane (1:1, v/v). Compound X (R_F 0.53) was located by spraying a test strip with Turnbull's blue solution (1% ferric chloride with 1% potassium ferricyanide [aqueous]), scraped from the plate, transferred to a small glass column and eluted with chloroform:methanol (1:1, v/v). The purity of the product was determined by GLC of a small sample after acetylation. From the IR, mass spectrometry and NMR spectra the structure of compound X was determined to be *trans*-(±)-3,4-bis[(3-hydroxyphenyl)methyl]dihydro-2-(3H)-furanone (HPMF, Fig. 3) on the following evidence.

The formula $C_{18}H_{18}O_4$ was established from the mass spectrum by the parent peak at $m/e = 298.1193$ (calculated, 298.1205) and prominent peaks at $m/e = 107.0503$ (calculated for C_7H_7O , 107.0497) and 108.0583 (calculated for C_7H_8O , 108.0575) were indicative of $-CH_2C_6H_4OH$ groups. The

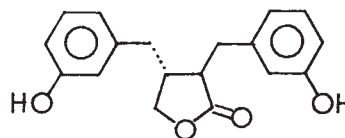


Fig. 3 Compound X; *trans*-(±)-3,4-bis[(3-hydroxyphenyl)methyl]dihydro-2-(3H)-furanone (HPMF).

presence of a γ -lactone (ν_{max} 1,770 cm⁻¹, C=O stretch) was evident from the IR spectrum. ¹³C-NMR revealed signals characteristic of two similar but non-equivalent disubstituted aromatic rings (δ 113.9, 114.0, 115.7, 116.3, 121.1, 121.7, 130.0 and 130.0, aromatic =CH—). In the ¹H-NMR (200 MHz) spectrum, the aromatic region showed the presence of two non-equivalent *m*-hydroxyphenyl groups. In addition, the AB-parts of two ABX-systems centred at δ 4.0 ($J_{AB} = 9$, $J_{AX} \approx J_{BX} \approx 7$) and δ 2.96 ($J_{AB} = 14$, $J_{AX} = 5$, $J_{BX} = 6$) could be detected, arising from the partial structures $-OCH_2-CH R-$ and $Ar CH_2CH-$.

The proposed structure (Fig. 3) is supported by ¹H-NMR data reported for closely related compounds and has been confirmed by synthesis (to be reported elsewhere)⁴. The racemic product (melting point 141–143 °C) was identical in all respects with the compound of natural origin. The absolute configuration and optical purity of the natural substance were determined by resolution of an intermediate (I) (Fig. 4) in the synthesis. The resolved compound (I) (Fig. 4) had $[\alpha]_D^{20} + 6.4^\circ$ (1% in chloroform) and optically active HPMF prepared from it had a melting point of 125–129 °C and $[\alpha]_D^{20} - 38.4^\circ$ (1% in chloroform). These values for the optical rotations compare favourably with reported values of $+4.8^\circ$ (ref. 5) and -34° (ref. 6) for the closely related compounds (II) and (III), respectively (Fig. 4). The specific optical rotations of samples of natural HPMF isolated from urine varied between 0° and -4° indicating an optical purity of $\leq 10\%$.

We have not detected HPMF in normal male urine and this, with the regularity of its cyclic excretion in women, leads to the conclusion that it is not of dietary origin. However, excretion of HPMF increases when anovulatory patients are treated with human menopausal gonadotropin. This and the pattern of its excretion during the menstrual cycle and in pregnancy suggest

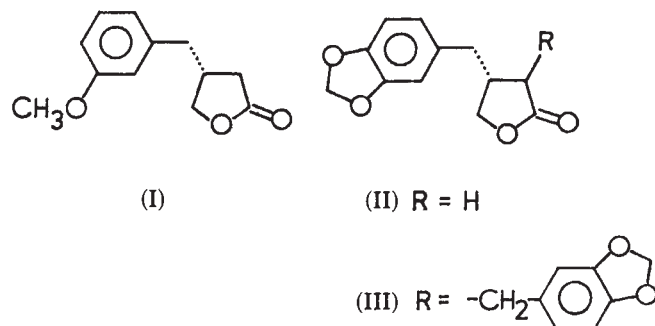


Fig. 4 Intermediate (I) and related compounds (II) and (III) in the synthesis of HPMF.

that there may be an ovarian source or stimulus for the production of HPMF. Investigations are now underway to establish the function and source of HPMF in the body and the biological properties of the synthetic substance. In this respect it is interesting that the same or a similar compound, and a substance with similar chemical and chromatographic properties seem to be excreted in the vervet monkey (*Cercopithecus aethiops pygerythrus*) during the reproductive cycle and in pregnancy².

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Lignans in man and in animal species

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In our laboratories, for several years, two phenolic compounds have been detected during gas chromatographic-mass spectrometric analysis of urinary steroid extracts from human and animal species. Although features of the mass spectra of their trimethylsilyl (TMS) ether derivatives resembled those of oestrogens, they were atypical of steroids. The possibility that they were artefacts of the isolation procedures was discounted after careful studies with blanks, by varying the extraction method and because they were present almost exclusively as conjugates of glucuronic acid. Several of the general characteristics of the unknown compounds were reported^{1,2} after one (referred to as compound 180/442) was found to have a cyclic pattern of excretion during the menstrual cycle of an adult vervet monkey (Fig. 1). An investigation of the nature and distribution of the compounds has shown them to be urinary constituents in humans, baboons, vervet monkeys and rats, and further related compounds have been detected, so far only in vervet monkey urine. We now report spectroscopic and chemical studies that show the two original compounds to be lignans, which have a 2,3-dibenzylbutane skeleton as their basic structure. Unlike all previously known natural lignans, invariably of plant origin, the two mammalian compounds carry phenolic hydroxy groups only in the *meta* position of the aromatic rings.

The two compounds are:

- (I) 2,3-bis-(3'-hydroxybenzyl)-butyrolactone (referred to in previous reports^{1,2} as compound 180/442).
 - (II) 2,3-bis-(3'-hydroxybenzyl)-butane-1,4-diol (referred to previously as compound 180/410).
- Some related compounds subsequently found in the urine of the vervet monkey correspond to I and II but have additional methoxy substituents in one or both of the phenolic rings:
- (III) 2-(3'-hydroxy-methoxybenzyl)-3-(3'-hydroxybenzyl)-butyrolactone.
 - (IV) 2-(3'-hydroxy-methoxybenzyl)-3-(3'-hydroxybenzyl)-butane-1,4-diol.
 - (V) 2,3-bis-(3'-hydroxy-methoxybenzyl)-butyrolactone.
 - (VI) 2,3-bis-(3'-hydroxy-methoxybenzyl)-butane-1,4-diol.

Several other compounds found in the urine of the vervet monkey appear from their physicochemical properties to be of related structure, but they have not been characterized.

The locations of the methoxyl groups in the phenolic rings of the relatively minor constituents (III-IV) remain to be established finally. In compound III the non-equivalence of the

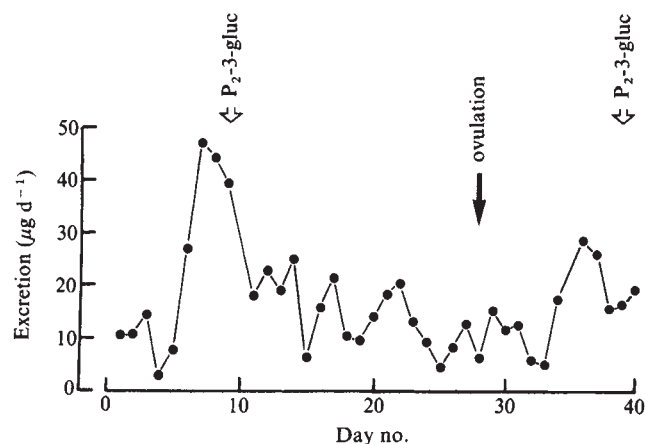


Fig. 1 Urinary excretion ($\mu\text{g d}^{-1}$) of 2,3-bis-(3'-hydroxybenzyl)-butyrolactone in a mature adult female vervet monkey (*Cercopithecus aethiops pygerythrus*) and its relationship to the time of ovulation and maximum corpus luteal activity determined from measurements of oestrone glucuronide and pregnadiol-3 α -glucuronide (P₂-gluc), respectively¹.

benzylic groups introduces uncertainty as to which aromatic ring contains the methoxyl function. Figure 2 illustrates the structural formulae of compounds I-VI. The evidence which led to the identification of I and II and the chemical synthesis which confirmed their structures are summarized here and will be described in detail elsewhere.

Mass spectra of the TMS ether derivatives of compounds I and II isolated from human urine and those of chemically synthesized 2,3-bis-(3'-hydroxybenzyl)-butyrolactone and 2,3-bis-(3'-hydroxybenzyl)-butane-1,4-diol are illustrated in Fig. 3; they are identical. Some aspects of the fragmentation pattern of these spectra have been discussed previously¹. The presence of the phenolic hydroxy group in the *meta* position is substantiated by the intense rearrangement ion at m/z 180. This ion is insignificant in TMS ether derivatives of *ortho* or *para* substituted lignan analogues and model compounds (work in preparation). The gas chromatographic (GC) characteristics of the TMS ether derivatives of the natural and synthetic compounds I and II on open-tubular glass capillary columns coated with silicone OV-1 were identical (retention times: (I, 27.50 methylene units (MU) and II, 27.70 MU). Accurate mass measurement of the molecular ions of the isolated compounds I and II showed their elemental composition to be $\text{C}_{18}\text{H}_{18}\text{O}_4$ and $\text{C}_{18}\text{H}_{22}\text{O}_4$ respectively in agreement with the structures shown in Fig. 2.

Nuclear magnetic resonance (NMR) spectra of the natural compounds I and II were compared with those of authentic

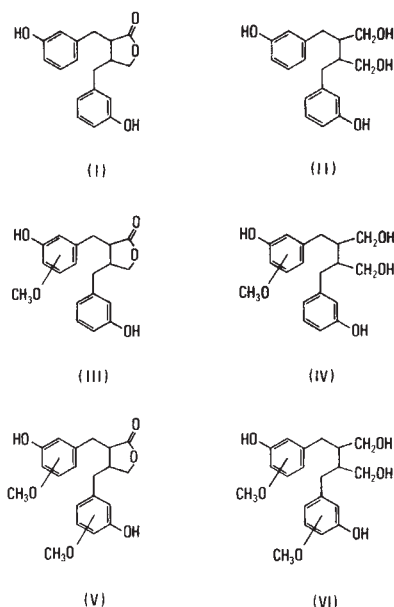


Fig. 2 Chemical formulae of newly identified lignans. Compounds I and II have been identified in the urine of humans, baboons, vervet monkeys and rats while III-VI have so far only been identified in vervet monkey urine³. All of these lignans have been identified in urine as glucuronide conjugates.

samples, respectively, of matairesinol dimethyl ether and dihydrocubebin (Fig. 4). The virtual identities of the high field regions of the pairs of spectra indicated the structures of the non-aromatic parts of the molecules I and II (Table 1). The aromatic regions of the spectra of I and II comprised complex multiplets split into two regions: a multiplet integrating for two protons, centred near 7.1 p.p.m., and a multiplet integrating for six protons, mainly between 6.6 and 6.8 p.p.m. A similar pattern found for some model *meta*-alkylated phenols indicated the location of the phenolic hydroxy groups in compounds I and II. The γ -lactone ring in compound I was further indicated by the infrared (IR) spectrum, with carbonyl absorption at $1,750\text{ cm}^{-1}$.

Compounds I and II were synthesized by an adaptation of the traditional route to lignans of the 2,3-dibenzylbutane type^{3,4}. A Stobbe condensation of 3-benzyloxybenzaldehyde (2 mol) with diethyl succinate (1 mol) gave bis-(3-benzyloxybenzylidene)-succinic acid. Catalytic hydrogenation, with simultaneous hydrogenolysis of the protecting benzyloxy groups, followed by reduction of the resulting 2,3-bis-(3'-hydroxybenzyl)-succinic acid with lithium aluminium hydride, and acidification, gave a mixture of products. Preparative thin layer chromatography (TLC) afforded samples which consisted essentially of 2,3-bis-(3'-hydroxybenzyl)-butyrolactone (I) and 2,3-bis-(3'-hydroxybenzyl)-butane-1,4-diol (II). The latter product appeared on the basis of GC and NMR data, to comprise a mixture of the *dl* and *meso* forms.

The TLC behaviour, and the IR spectra of the synthetic and natural compounds I and II, and the NMR spectra of the two samples of the dibenzylbutyrolactone (I), were indistinguishable. The NMR spectrum of the synthetic dibenzylbutanediol (II) contained all the features in the spectrum of the natural material, and some additional signals attributed to the presence of the *meso* isomer.

Compound I is of greatest interest at present because it is quantitatively the most important in human urine, plasma and bile and its daily urinary excretion is comparable to that of many steroid metabolites^{2,5}. It is the most likely precursor of most of the other compounds in the series and can be reduced chemically to II. As indicated earlier it is present principally as a glucuronide conjugate²², as are the other lignans identified in the series.

We found that the urinary excretion of the dibenzylbutyrolactone (I) increased significantly during the mid-luteal phase of the menstrual cycle and showed a marked surge in early pregnancy in humans^{2,5} as well as the vervet monkey¹. In four women the urinary excretion of I was at its minimum at the time of ovulation and then increased, reaching a maximum during the mid-luteal phase (Fig. 5). Furthermore, during pregnancy maximum excretion of dibenzylbutyrolactone occurs between weeks 14 and 22 of gestation^{2,5}. A non-steroidal compound has been reported⁶ to show similar urinary excretion patterns during the human menstrual cycle and in pregnancy.

The biosynthetic pathway of the lignans is not yet known, although their aromatic nature might suggest a dietary origin or formation by intestinal bacteria. Preliminary results, however, indicate that they may be ovarian in origin or that their production is influenced by ovarian function. For instance, in addition to the cyclic behaviour of compounds I and II⁵, their excretion in a limited number of postmenopausal women studied was lower than in normally ovulating women, while in three ovariectomized/postmenopausal women excretion was approximately 5–10% of that in normal ovulating women. Because the compounds are barely detectable in the urine of newborn infants it seems that in pregnancy they are essentially maternal in origin.

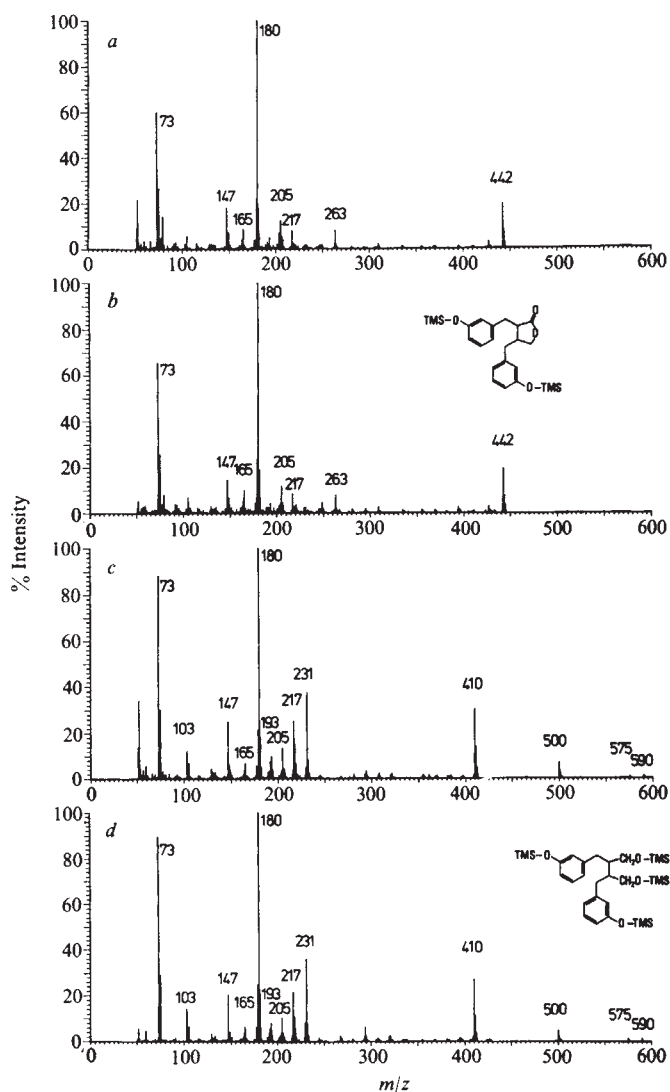


Fig. 3 Mass spectra of the TMS derivatives of: a, natural compound I; b, synthetic 2,3-bis-(3'-hydroxybenzyl)-butyrolactone; c, natural compound II and d, synthetic 2,3-bis-(3'-hydroxybenzyl)-butane-1,4-diol.

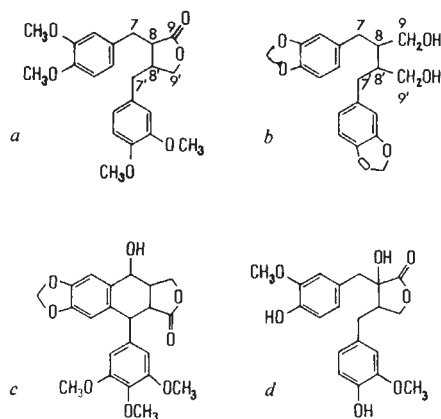


Fig. 4 Chemical structure of the lignans: a, matairesinol dimethyl ether; b, dihydrocubebin; c, podophyllotoxin; d, wikstromol.

Compounds I and II were also found in adult males where their urinary excretion is quantitatively similar to that in the follicular phase of normal ovulating women. Although we have no information on the site of their production in men, the fact that they are almost undetectable in the urine of newborn infants^{2,5} and in extremely low concentrations in children of both sexes suggests that their production is related to gonadal development.

The existence of lignans in humans, and preliminary observations on their disposition, pose questions as to their phy-

Table 1 ¹ H NMR spectra at 100 MHz in CDCl ₃			
Compound	H-7,7*	H-8,8*	H-9' or H-9,9'
2,3-bis-(3'-hydroxybenzyl)-butyrolactone(I)	2.56 (8)	2.94 (9)	4.0 (about 34)
Matairesinol dimethyl ether	2.55 (8)	2.94 (9)	4.0 (about 30)
2,3-bis-(3'-hydroxybenzyl)butane-1,4-diol (II)	2.68 (10)	2.02 (about 10)	3.6-3.7 (br.m.)
Dihydrocubebin	2.66 (12)	1.82 (about 14)	3.63 (br.m.)

Δ Values are given in p.p.m. relative to the Me₄Si; W_{1/2} is given in parentheses. The assignments of spectra are tentative: a detailed analysis of NMR spectra is in progress and will be published elsewhere. br.m., Broad multiplet.

* Numbering system in accordance with that used in ref. 7 (see also Fig. 4).

siological and biological significance. The lignans are uncommon plant constituents which have been found so far only in higher plants of the orders Gymnospermophytina and Angiospermophytina. They have been investigated extensively by natural product chemists and various types of dibenzylbutane and related structures have been catalogued^{7,8}. As far as we know, however, compounds with the substitution pattern reported here have not been identified in any plant species, presumably reflecting different pathways of biosynthesis or metabolism.

Lignans have been classified by the US National Cancer Institute^{9,10} to be of 'high interest' as potential anticancer agents and several have been used in clinical trials to treat tumours¹¹⁻¹⁴. Podophyllotoxin¹⁵ (Fig. 4) is probably the best known lignan; it is derived from the roots of the Mayapple plant of the central USA. This lignan and a number of other less complex natural (for example, wikstromol, Fig. 4) and synthetic lignans, exhibits anti-mitotic activity and has been effective in treating several types of tumour in animals¹⁶⁻²⁰. Whether or not the lignans of humans have similar activity remains to be determined although it is interesting that to be biologically active the plant compounds require a *trans* configuration of the benzyl substituents on the C₂-C₃ of the butane.

The diphenolic nature of compounds I-VI resemble many of the substances known either to exert oestrogenic action or to block oestrogen receptor sites²¹. The increased urinary excretion of the dibenzylbutyrolactone (I) in the luteal phase, a conspicuous feature of the menstrual cycles studied so far, suggests that this lignan, or a related compound, may be associated with luteolytic activity and the regulation of the length of the luteal phase of the cycle.

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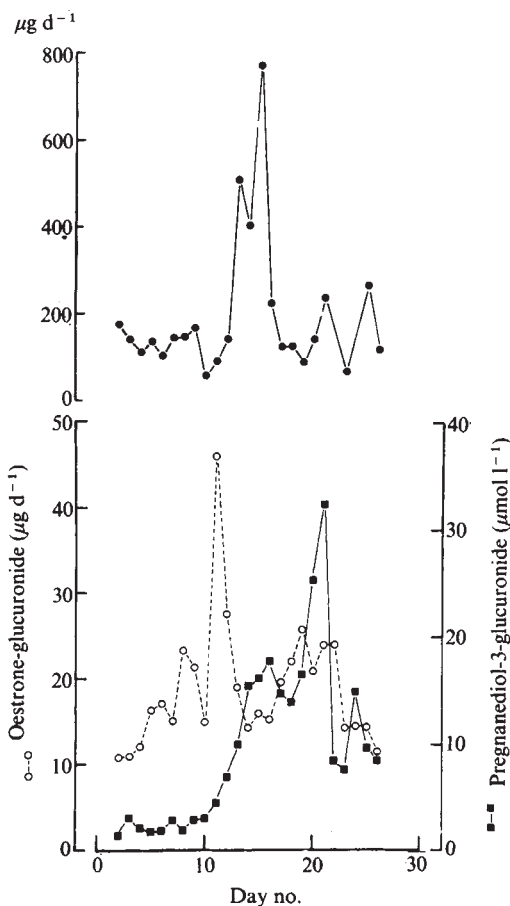


Fig. 5 Urinary excretion ($\mu\text{g d}^{-1}$) of 2,3-bis-(3'-hydroxybenzyl)-butyrolactone (upper) during the menstrual cycle of a normal ovulating woman. Oestrone-glucuronide and pregnanediol-3 α -glucuronide were measured by radioimmunoassay and indicate the ovulatory and luteal phases of the cycle.

Endogenous digitalis-like substance in plasma of volume-expanded dogs

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Considerable evidence supports the hypothesis that renal sodium excretion is partly regulated by a humoral agent released or activated by extracellular fluid volume (ECFV) expansion¹, including the demonstration of biological activities in extracts of plasma and urine which support the natriuretic hormone (NH) hypothesis²⁻⁴. NH is a low molecular weight peptide formed from a precursor in plasma⁵, which inhibits sodium transport (anti-natriuretic activity) in isolated toad bladder, an analogue of the distal renal tubule and collecting duct. Other evidence indicates that NH can inhibit renal $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ ⁶, which suggests that it might be an endogenous digitalis-like substance. Although an immunoreactive ouabain-like substance has been demonstrated in amphibian plasma⁷, none has been reported in mammals. To identify an endogenous digitalis-like substance, we used an approach suggested by the work of Spector and his collaborators, who demonstrated that biological substances which bind to the same receptors as drugs, such as endogenous opioids⁸ and benzodiazepines⁹, may compete with antibodies specific for the drugs. Hough and Edwardson¹⁰ showed that antibodies to thaumatin, a sweet-tasting plant protein, also bind non-protein sweet substances in a linearly related fashion to the sweetness of the compound, suggesting that the antibody binding site may be similar to the sweet taste receptor. Based on these observations, we postulated that if NH were an endogenous digitalis-like substance, it might bind to antibodies specific for digitalis glycosides. We report here the preliminary isolation of a substance in plasma of dogs which competes with digoxin for two specific digoxin antibodies and is an inhibitor of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ activity. Increased amounts of this factor are found in plasma of volume-expanded dogs, suggesting it is the putative NH.

Blood was obtained from pentobarbital-anaesthetized, saline-expanded and hydropenic dogs. The procedures for acute saline expansion and the protocol for plasma processing have been published previously⁵. Deproteinized plasma extracts, representing an original plasma volume of 10 ml, were eluted by Diafiltration through Amicon UM-10 membranes (exclusion limit <10,000 molecular weight) with 0.05 M acetic acid. Four volumes per sample were eluted, freeze-dried and further purified by HPLC.

Figure 1a shows the chromatographic pattern obtained when a deproteinized plasma diafiltrate from a volume-expanded dog was chromatographed on a Partisil SCX (strong cation exchange) column (Whatman). Because we had previously shown that the anti-natriuretic factor believed to be the active principle of NH eluted in the void volume on SCX⁵, this fraction was tested for immunoreactivity with anti-digoxin antibodies. A substance showing binding to two anti-digoxin antibodies was demonstrated (Fig. 2). The dilution curve of the endogenous immunoreactive substance was similar to that of unlabelled digoxin standard. The two antibodies used were produced in different species to digoxin conjugated to either bovine serum albumin or human serum albumin. The apparent concentration of the endogenous substance in the SCX I sample shown in Fig. 2 was 355 pg digoxin equivalents per ml of plasma.

The SCX void volume fraction was further purified on reverse-phase chromatography (Lichrosorb-ODS; Fig. 1b). Salts emerged in the void volume followed by several, distinct

fluorescamine-positive peaks. Each tube after the salt peak was assayed for immunoreactivity to the goat anti-digoxin antibody, and for $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ inhibitory activity with a purified hog brain ATPase preparation (Fig. 3). Immunoreactivity and ATPase-inhibitory activity eluted in two distinct peaks, the first coming immediately after the salt. These peaks of biological activity and immunoreactivity eluted at similar times, and corresponded to two distinct fluorescamine-positive peaks on ODS chromatography (Fig. 1b). Similar elution patterns were obtained with plasma extracts from both hydropenic and saline-expanded dogs. Most importantly, however, activity was higher in saline-expanded plasma samples. ATPase-inhibitory activity at the maximum of peak 1 from four saline-expanded plasma extracts was 185 ± 46 pmol ouabain equivalents (equiv.) per tube compared with 66 ± 25 pmol ouabain equiv. per tube at the maximum of peak 1 from four hydropenic dog samples ($P < 0.03$). Similarly, at the maximum of peak 2, ATPase-inhibitory activity was 148 ± 43 pmol ouabain equiv. per tube in four samples from saline-expanded dogs and 43 ± 26 pmol ouabain equiv. per tube in four samples from hydropenic dogs ($P < 0.03$). Comparison of peak heights indicates that saline-expanded samples are three times more active than hydropenic samples, but total differences are actually greater. Because the total amount of biological activity in the original samples is equal to the area under the curve for each peak, and because each sample has two approximately equal peaks, the total difference between the two groups would be the sum of the differences for each peak.

The quantitative difference in digitalis-like activity obtained between the radioimmunoassay (RIA) and the ATPase activity is of interest (Fig. 3). In the sample shown in Fig. 3, immunoreactivity of peak 1 was 480 fmol digoxin equiv. per tube, whereas the ATPase inhibitory activity was 163 pmol ouabain equiv. per tube. As RIA is a function of numbers of molecules, it probably reflects the actual concentration of the endogenous digitalis-like substance more accurately. This indicates that the endogenous

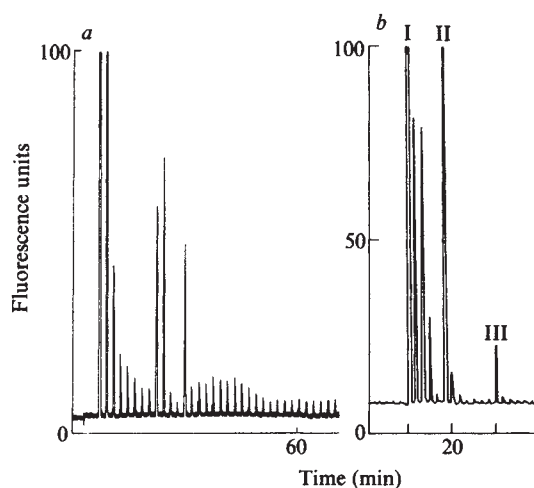


Fig. 1 *a*, Freeze-dried diafiltrate representing 10 ml of plasma was suspended in 400 μl of 10% formic acid and separated on Partisil SCX with a pyridine-acetate gradient¹¹. The column flow rate was 400 $\mu\text{l min}^{-1}$. The column effluent was monitored by a preparative fluorescamine monitoring system¹² which allows detection of primary amines (including peptides and amino acids) by automated, discontinuous sampling of the column effluent. Approximately 1–2% of the effluent was used for detection while the remainder was collected in a fraction collector at 4-min intervals. The discontinuous appearance of the chromatogram is a result of the monitoring system with each line representing one sampling period (2-min interval). The void volume peak (12–20 min), which contains all the plasma salts, was tested for immunoreactivity (Fig. 2) and purified further by reverse phase chromatography. *b*, Reverse-phase chromatography was performed on Lichrosorb ODS. The sample was eluted isocratically in 0.5 M pyridine titrated to pH 3 with formic acid, with a column flow rate of approximately 200 $\mu\text{l min}^{-1}$. Fractions were monitored and collected every 2 min. The salt emerged in the void volume (ODS-I) and the next two fractions (ODS-II and ODS-III) were tested for immunoreactivity and $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ inhibitory activity.

substance is about three orders of magnitude more potent an inhibitor of purified brain ($\text{Na}^+ + \text{K}^+$)ATPase than ouabain. This finding is in accord with our observation that 10^{-5} M ouabain is necessary to produce the same degree of antinatriuretic activity (25% inhibition of short-circuit current) in toad bladder as that produced by an extract of 5 ml of plasma from volume-expanded dogs (W. C. Plunkett *et al.*, in preparation).

As a further demonstration that the digitalis-like immunoreactive substance is identical to the ATPase inhibitor, preliminary studies have shown that immunoprecipitation of plasma diafiltrates with the goat anti-digoxin antibody yields two peaks on ODS chromatography which are similar to those

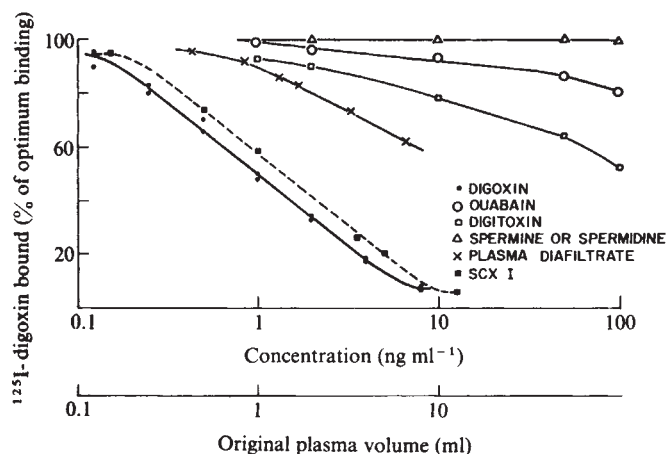


Fig. 2 Radioimmunoassay was performed on SCX fraction I and diafiltrates reconstituted in 500 μl of distilled water, adjusted to pH 7.6 with NaOH. Duplicate dilutions of these samples were assayed for cross-reactivity to either an ^{125}I -digoxin clinical radioimmunoassay kit (NEN) or an RIA for digoxin developed in our laboratories using a goat anti-digoxin antibody. The NEN antibody is produced in rabbits by immunization with a digoxin-human serum albumin conjugate, and shows less than 5% cross-reactivity with digitoxin, and essentially none with ouabain or any other steroid glycoside. For assay with the NEN kits, the antibody was incubated with samples or standards in the presence of ^{125}I -digoxin according to procedures provided. Separation of bound antigen from free antigen was accomplished either by charcoal adsorption, or by precipitation with a second antibody (sheep anti-rabbit γ -globulin). Although both separation techniques provided similar results, the double antibody precipitation technique was used routinely. A second, more sensitive RIA developed in our laboratories using an antibody produced in goats immunized with a digoxin-bovine serum albumin conjugate is demonstrated. The degree of cross-reactivity of this antibody with digitoxin, ouabain, spermine or spermidine (known ATPase inhibitors) is shown. Thyrotropin-releasing hormone and various amino acids including phenylalanine and proline were also tested and showed no cross-reactivity. For this RIA the anti-digoxin antibody was incubated for 2 h at 37°C , pH 7.6 (phosphate-buffered saline) in the presence of ^{125}I -digoxin (Burroughs Wellcome) and duplicates of either samples or standards. Rabbit anti-goat γ -globulin was then added to the reaction mixture and incubated for an additional hour. Samples were then centrifuged at 3,000 r.p.m. for 30 min, the supernatant decanted and the pellet counted in a γ counter. Per cent bound was calculated by subtracting nonspecific background and comparing the results with 100% for optimum binding (tubes containing reaction mixture but no unlabelled standard or unknown sample). This immunoassay technique yielded a 10-fold increase in sensitivity over that of the clinical assay kits. Further details on this method will be presented elsewhere. The effect of various glycosides and polyamines on binding of ^{125}I -digoxin to its specific antibody is also shown. Aliquots (100 μl) of standard solutions of glycosides or polyamines were added to the reaction mixture containing buffer, ^{125}I -digoxin and antibody for a total volume of 400 μl . Concentrations of standards are calculated as ng ml^{-1} solution and plotted according to the concentration of the original stock solution. Aliquots (100 μl) of plasma extract from SCX I (void volume lyophilized, reconstituted in H_2O , pH 7.6 with NaOH) corresponding to plasma volumes ranging from 0.15 to 12.0 ml were also tested for their ability to compete with ^{125}I -digoxin for its antibody. The binding curve for SCX I is more nearly parallel to the digoxin standard than is the binding curve from the diafiltrate. Apparently, this is due to the removal of interfering substances during the purification procedure. Spermine or spermidine at 100 ng ml^{-1} show no cross-reaction with the antibody whereas similar concentrations of ouabain and digitoxin each show cross-reactivity of less than 1%. Points represent the mean of duplicates which show a variance of less than 5%. The data for plasma diafiltrates and SCX I samples are plotted as a function of original plasma volume; all other substances shown are plotted by concentration.

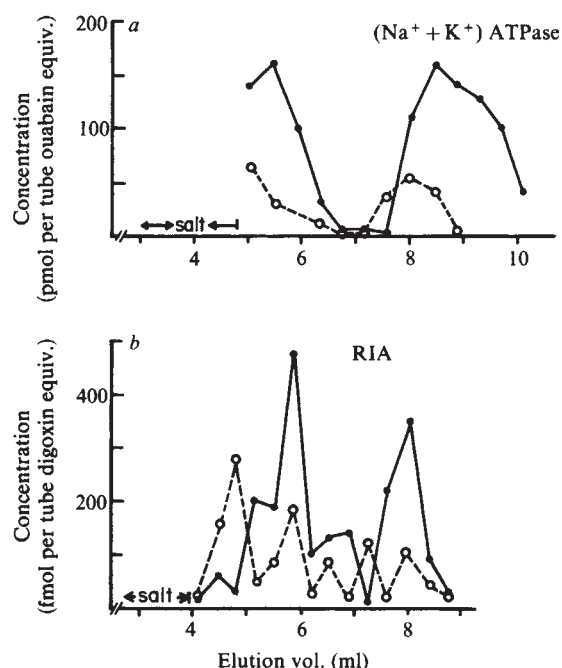


Fig. 3 Assays for ($\text{Na}^+ + \text{K}^+$)ATPase inhibitory activity (a) and digoxin immunoreactivity (b) were performed on each 2-min fraction (400 μl) of ODS column effluent. The solid line represents volume-expanded samples, while the dotted line represents hydroponic samples. ATPase inhibition was assayed using a purified ($\text{Na}^+ + \text{K}^+$)ATPase enzyme derived from hog cerebral cortex (Sigma). This enzyme was originally used in clinical assays for digoxin and digitoxin¹³, and has also been used to assay for NH_4^+ . Ouabain or lyophilized HPLC samples were dissolved in an imidazole-HCl buffer (10 mM, pH 7.2) containing sodium (100 mM), potassium (5 mM), magnesium (1 mM) and EGTA (0.5 mM). After addition of enzyme solution the samples were incubated for 10 min in a shaking water bath at 37°C . ATP was added to a final concentration of 1 mM. The total volume of each incubation tube was 1 ml. Blank tubes contained only ATP and the buffered ion solution, while control ATPase activity was determined in the absence of ouabain. After 15 min of incubation in the presence of ATP the reaction was stopped by the addition of 0.7 ml of cold 13% TCA. ATPase activity was determined as the amount of inorganic phosphate liberated by the method of Eibl and Lands¹⁵. The minimum sensitivity of this assay is approximately 10 pmol per ml of ouabain, while 100 nmol causes 100% inhibition of enzyme activity. The assay system was standardized with ouabain rather than digoxin because solubilization of digoxin requires an organic solvent which interferes with the assay. Although it is possible to aliquot and steam-dry digoxin¹³, we preferred to avoid this rather cumbersome procedure. A dose-response curve for ouabain was generated for each set of unknowns and values for each unknown were calculated from the standard curve by computer program, and expressed as ouabain equivalents.

shown in Fig. 1b. Assay for ATPase inhibitory activity of these two peaks showed a similar difference between samples from volume-expanded and hydroponic dogs as shown in Fig. 3.

It is not clear from these studies why two peaks are recovered. However, we have previously reported two fluorescamine-positive peaks on ODS chromatography of the SCX void volume, both of which showed a high degree of correlation with anti-natriuretic activity⁵. Thus, there appear to be two molecular species with similar biological activity that differ somewhat in their basic structure.

The evidence presented here suggests that the digitalis-like factors are peptides, as they can be separated by chromatographic protocols for small peptides, and elute in fluorescamine-positive peaks. In addition, we have found that treatment with concentrated HCl at room temperature for 30 min destroys all activity. This susceptibility to acid hydrolysis indicates that the ATPase inhibitory activity is not due to known endogenous ATPase inhibitors such as polyamines¹⁶, vanadium¹⁷ or bufotoxin-like steroids¹⁸. The initial studies on the use of Partisil SCX with biological samples¹¹ also demonstrated longer retention times for polyamines than is seen for SCX I.

Interestingly several investigators have reported data which in retrospect support our observations. Early attempts to measure

cardiac glycosides in plasma by the red blood cell ^{86}Rb uptake assay noted an endogenous interfering substance in patients with congestive heart failure or renal failure¹⁹, which could be separated from cardiac glycosides by its water solubility²⁰. The same enzyme preparation used in these studies was originally developed as an assay for digoxin by Burnett and Conklin¹³, who also noted endogenous digoxin-like activity in normal non-digitalized controls, and in patients with fluid balance problems¹³. More recently, it has been reported²¹ that RIAs for digoxin in patients with renal failure tend to overestimate plasma levels when compared with an HPLC method for the drug. If the factor demonstrated in our studies is the endogenous substance which interferes with clinical assays for digoxin, then its plasma level seems to be elevated in some pathological conditions.

We should mention that the observed plasma levels of endogenous digoxin-like factor would be toxic to an intact animal. This apparent discrepancy could be explained if NH circulated as an inactive precursor, as suggested previously by us⁵. As noted, the plasma isolation protocol used in this study includes a period of incubation to allow precursor breakdown. Alter-

natively, *in vivo* receptor binding of the endogenous substance for $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ could be much lower for all organs other than the target organ (presumably the kidney).

Thus, for the first time we have demonstrated and partially purified a circulating factor in mammals which is an inhibitor of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ and possesses a structure which is recognized by antibodies to the cardiac glycoside digoxin. We have suggested that an appropriate name for this factor would be 'endoxin'²². Endoxin has a similar HPLC retention time as the previously demonstrated anti-natriuretic factor⁵, and both endoxin and the anti-natriuretic factor are present in higher concentrations in volume-expanded than in hydropenic subjects. These data support the hypothesis that these several biological and immunoreactive activities are due to the same factor, that this substance is the putative NH, and that anti-digoxin antibodies can be used as an RIA for NH. Confirmation of this hypothesis will require the biochemical characterization of endoxin, which is now in progress.

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Unusual levels of (ADP-ribose)_n and DNA synthesis in ataxia telangiectasia cells following γ -ray irradiation

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Poly (ADP-ribose) polymerase is a eukaryotic chromosomal enzyme which utilizes the ADP-ribose moiety of NAD to synthesize the nucleic acid homopolymer (ADP-ribose)_n (ref. 1). The precise function of (ADP-ribose)_n has not been fully established although it does covalently modify chromosomal proteins by ADP-ribosylation^{2,3}. Here we demonstrate that γ -ray irradiation of lymphoblastoid cells from normal subjects results in depressed DNA synthesis and increased (ADP-ribose)_n synthesis. Irradiation of lymphoblastoid cells from patients with the autosomal recessive disease ataxia telangiectasia (AT), however, failed to depress DNA synthesis and did not elevate (ADP-ribose)_n levels. We have confirmed that (ADP-ribose)_n is synthesized in response to DNA damage and we propose that this polymer may function in the recovery from DNA damage by suppressing DNA synthesis.

The synthesis of (ADP-ribose)_n is increased by a variety of agents which cause DNA damage. Radiation⁴⁻⁷, alkylating agents^{8,9} and bleomycin, a radiomimetic antibiotic, increase (ADP-ribose)_n synthesis and DNA repair synthesis in normal human lymphocytes¹⁰. The level of (ADP-ribose)_n increases in normal lymphoblastoid cell lines after UV-irradiation, whereas this response is either absent or delayed in xeroderma pigmentosum (XP) cells¹¹, which are defective in the removal of UV-induced thymine dimers^{12,13}. The synthesis of (ADP-

ribose)_n is increased in proportion to the number of DNA strand breaks induced by exogenous endonucleases⁴.

We have investigated the synthesis of (ADP-ribose)_n in lymphoblastoid cell lines from two ataxia telangiectasia (AT) patients. Patients with this disorder show a predisposition to cancer and enhanced sensitivity to X-ray or γ -ray irradiation as measured by colony survival¹⁴.

Lymphoblastoid cell lines from two normal subjects (LIZ, DEW) and two AT patients (GM1525/AT2BI, GM1526/AT8BI) were irradiated with γ -rays (0-1,000 rad) at 4 °C. The synthesis of (ADP-ribose)_n from both normal subjects increased in proportion to γ -ray dose. At a dose of 1 krad the synthesis of (ADP-ribose)_n increased by 54-58% over mock irradiated normal cells. Lymphoblastoid cell lines from both AT patients showed a smaller (8-14%) increase in (ADP-ribose)_n following the same dose of γ -rays (Fig. 1).

In all experiments the acid-insoluble product synthesized from ¹⁴C-NAD was shown to be (ADP-ribose)_n by the inability of DNase I (100 $\mu\text{g ml}^{-1}$) and RNase I (500 $\mu\text{g ml}^{-1}$) to hydrolyse the product, whereas 91% was hydrolysed into acid-soluble products by 600 $\mu\text{g ml}^{-1}$ snake venom phosphodiesterase (Table 1).

To investigate the possibility that poly (ADP-ribose) polymerase was deficient in AT cells, lymphoblastoid cell lines from a normal subject (LIZ) and an AT (GM1526/AT8BI) patient were permeabilized¹⁵ and treated with exogenous DNase I (100 $\mu\text{g ml}^{-1}$) and the synthesis of (ADP-ribose)_n measured. Both the normal and AT cell lines synthesized (ADP-ribose)_n in similar amounts in response to DNA damage introduced by exogenous DNase I (Table 1). This suggests that the inability of AT cells to synthesize (ADP-ribose)_n was not due to an intrinsic defect in poly (ADP-ribose) polymerase.

It has been reported that NAD the substrate for (ADP-ribose)_n synthesis caused decreased ³H-TTP incorporation and template activity of chromatin in isolated rat liver nuclei^{16,17} whereas this decrease was not observed in nuclei from

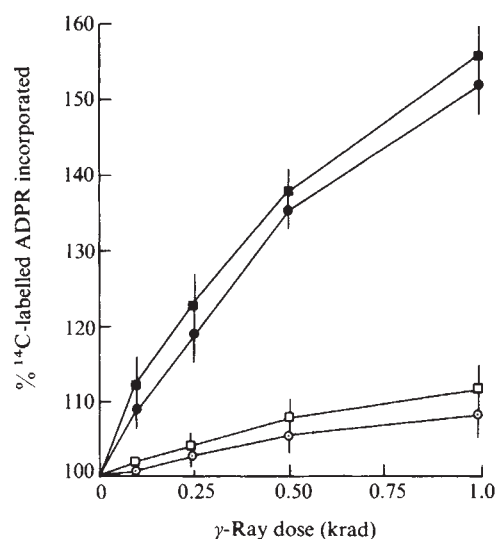


Fig. 1 Effect of increasing γ -ray irradiation dose on (ADP-ribose)_n synthesis in permeable lymphoblastoid cell lines from normal subjects (LIZ ■, DEW ●) and AT patients (GM1525/AT2BI □; GM1526/AT8BI ○). Cell suspensions (7.8×10^5 cells per ml) in exponential growth (72 h) were resuspended in RPMI medium supplemented with 10% (v/v) fetal calf serum (FCS) and irradiated at 4 °C using a ^{60}Co γ -ray source at a dose rate of 200 rad min⁻¹. Irradiated and nonirradiated cell suspensions were centrifuged at 2,000g for 5 min, washed twice in Dulbecco A phosphate-buffered saline (containing 1.15 g l⁻¹ Na₂HPO₄, 0.2 g l⁻¹ KH₂PO₄, 0.2 g l⁻¹ KCl and 8 g l⁻¹ NaCl at pH 7.3), resuspended in permeabilizing buffer pH 7.8 (containing 10 mM Tris, 1 mM EDTA, 4 mM MgCl₂, 1 mM dithiothreitol and 0.05% (v/v) Triton X-100) for 15 min at 4 °C. 100 μ l of cell suspensions were incubated at 30 °C with 10 μ l of ^{14}C -NAD (final concentration 5 μ l ml⁻¹, 100 μ M) in the presence of 50 μ l of assay buffer pH 7.8 (containing 50 mM Tris, 15 mM MgCl₂, 15 mM KCl, 10 mM NaF and 3 mM cyclic AMP). Triplicate samples were incubated for 10 min. The reaction was stopped with 0.2 ml of 20% (w/v) trichloroacetic acid (TCA). The samples were maintained at 4 °C for 2 h, filtered onto glass-fibre disks (Whatman GF/C) and washed with 10% (w/v) TCA containing 2% (w/v) Na₄P₂O₇ (5 $\times$ 10 ml). Protein²³ was determined and radioactivity on the filters was measured in Insta-gel (Packard). Enzyme activity (d.p.m. per μ g of protein) is expressed as a percentage of the activity in mock-irradiated cells. Results are presented as the mean $\pm$ s.e.m. of three experiments each performed in triplicate.

lymphoma¹⁸ and hepatoma¹⁹ cells. Poly (ADP-ribose) polymerase activity is inversely related to DNA synthesis in rat cardiac muscle, and the synthesis of (ADP-ribose) has been implicated in the regulation of DNA synthesis²⁰. We therefore measured DNA synthesis in these normal and AT lymphoblastoid cell lines in response to γ -ray irradiation. As previously demonstrated²¹ an inhibition of [Me -³H]thymidine incorporation into the DNA of both normal cell lines (LIZ, DEW) after treatment of cells with a γ -ray dose of 500 rad was observed (Fig. 2). However, no inhibition of [Me -³H]thymidine incorporation into the DNA of either AT cell line (GM1525/AT2BI, GM1526/AT8BI) was observed at a γ -ray dose of 500 rad (Fig. 2). Control experiments demonstrated that the amount of [Me -³H]thymidine transported into both normal and AT lymphoblastoid cells decreased by similar amounts after treatment with γ -rays.

These results confirm that γ -ray irradiation produces an inverse relationship between (ADP-ribose)_n and DNA synthesis in normal human lymphoblastoid cells. It has been proposed that suppressed DNA synthesis and stimulation of (ADP-ribose)_n are associated with the development of DNA strand breaks²². If this is correct the absence of an inverse relationship between (ADP-ribose)_n and DNA synthesis in the two AT lines suggests one of at least two possible alternatives. First, the AT cells may have a lower number of strand breaks;

Table 1 Characterization of the acid-insoluble product synthesized from ^{14}C -NAD in permeable DNase I-treated lymphoblastoid cells from a normal subject (LIZ) and an AT patient (GM1526/AT8BI)

Treatment	Acid-insoluble radioactivity remaining after treatment with nuclease (d.p.m. per mg of protein)	
	Normal cells	Ataxia telangiectasia (GM1526/AT8BI)
100 °C control	12,922 $\pm$ 646 (3)	19,100 $\pm$ 947 (3)
Deoxyribonuclease (100 μ g ml ⁻¹)	13,164 $\pm$ 661 (3)	19,100 $\pm$ 995 (3)
Ribonuclease (500 μ g ml ⁻¹)	12,320 $\pm$ 610 (3)	18,038 $\pm$ 879 (3)
Snake venom phosphodiesterase (600 μ g ml ⁻¹)	1,246 $\pm$ 58 (3)	1,380 $\pm$ 67 (3)

Cells (7.8 – 8.3×10^5 cells per ml) in exponential growth (72 h) were made permeable as described in Fig. 1 legend. Cell suspensions (4 ml) were incubated in the presence of DNase I (100 μ g ml⁻¹) for 30 min at 37 °C. Aliquots (50 μ l) were removed to measure total (ADP-ribose)_n synthesis in 100 μ l of poly (ADP-ribose) polymerase assay buffer, pH 7.8 and 10 μ l ^{14}C -NAD added. Reactions were terminated at 100 °C for 5 min. Aliquots (0.3 ml) of the DNase I treated cell suspensions heated at 100 °C for 5 min were incubated at 37 °C for 30 h in the presence of DNase I (100 μ g ml⁻¹), RNase I (500 μ g ml⁻¹) or snake venom phosphodiesterase (600 μ g ml⁻¹). Reactions were terminated with 0.2 ml of 20% (w/v) TCA containing 2% (w/v) Na₄P₂O₇. Acid-insoluble product was collected and radioactivity counted as described in the legend to Fig. 1. Results are presented as the mean $\pm$ s.e.m. of three experiments each performed in triplicate.

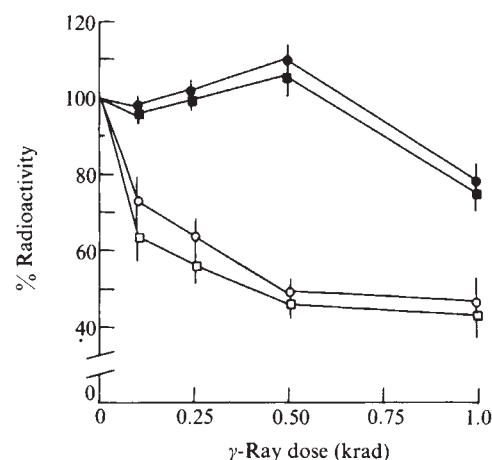


Fig. 2 Effect of increasing γ -ray radiation dose on DNA synthesis in lymphoblastoid cell lines derived from normal subjects (LIZ □, DEW ○) and AT patients (GM1525/AT2BI ■, GM1526/AT8BI ●). Cells in exponential growth (72 h) were resuspended in RPMI medium (7.4 – 8.3×10^5 cells per ml) supplemented with 10% (v/v) FCS and irradiated at 4 °C at the indicated dose using a ^{60}Co γ -ray source at a dose rate of 200 rad per min. Irradiated and non-irradiated cell suspensions were incubated after irradiation for 30 min at 37 °C and then 10 μ l of [Me -³H]thymidine (final concentration 10 μ Ci ml⁻¹, 10 μ M) added. Triplicate tubes were incubated for 30 min at 37 °C. Cells were collected and washed twice in Dulbecco A phosphate-buffered saline, pH 7.3 at 4 °C (2×20 ml). Cells were resuspended in 1 ml of a buffer (containing 50 mM Tris, 4 mM MgCl₂ and 0.2 M NaCl at pH 7.4) and 0.2 ml of 20% (w/v) TCA added. The acid-insoluble product was washed in 10% (w/v) TCA (5×10 ml) and dissolved in 1 ml of 0.1 M NaOH. Protein was measured and radioactivity determined by liquid scintillation counting in Insta-gel. DNA synthesis (d.p.m. per μ g of protein) in γ -ray irradiated cells is expressed as a percentage of mock irradiated (100%) control cells. Results are presented as the mean $\pm$ s.e.m. of four experiments each performed in triplicate.

there is, however, no evidence to support this²⁴. Second, it might suggest that the damage produced by γ -rays in these cells is not translated into a signal which stimulates poly (ADP-ribose) polymerase. The synthesis of (ADP-ribose)_n may have a role in the protection of DNA by suppressing DNA synthesis during states of DNA damage, and AT cells are hypersensitive to the effects of ionizing radiation for this reason.

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Defective DNA repair and increased lethality in ataxia telangiectasia cells exposed to 4-nitroquinoline-1-oxide

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Ataxia telangiectasia (AT) is a rare autosomal recessive disorder in man characterized by progressive loss of muscular coordination (due to neurodegeneration) and permanent dilation of the small blood vessels of the eyes and skin^{1,2}. AT patients also have repeated sinopulmonary infections, immune defects associated with thymus underdevelopment, and abnormal endocrine functions^{1,2}. Affected patients are at high risk of developing malignancy, particularly lymphomas and lymphatic leukaemias¹, and there are clinical indications^{3–5} that AT patients are hypersensitive to conventional radiotherapy administered for treatment of malignancy. Cultured diploid fibroblasts from AT donors are consistently hypersensitive to ionizing radiation^{2,6,7}, apparently due to defective enzymatic repair of radiogenic DNA damage^{2,8}. We have determined the survival responses and DNA repair abilities of AT cells exposed to 4-nitroquinoline-1-oxide (4NQO), a chemical carcinogen whose DNA-damaging properties partially mimic those of ionizing radiation⁹. We report here that certain AT cell strains show hypersensitivity to inactivation by 4NQO and defective repair of 4NQO-induced adducts in DNA.

The mutant strains studied (Table 1) were selected on the basis of their *in vitro* DNA repair characteristics. AT2BE and AT3BI are defective in the excision repair of γ -ray-induced DNA base damage^{2,8}; this is manifested in reduced levels of γ -ray-induced repair replication. The repair defects in AT2BE and AT3BI are genetically distinct, since the strains have been assigned to different complementation groups in cell fusion studies¹⁰. In the strain AT4BI^{2,10}, no anomalous repair of radiogenic base damage has been detected but it is possible that this strain is defective in the repair of a class of radiogenic DNA lesions that is simply not discernible in the repair assays used². XP9RO was derived from a patient with the sunlight-sensitivity disorder xeroderma pigmentosum (XP). This strain (defective in the repair of UV-induced pyrimidine dimers in DNA^{11,12}) is utilized as a sensitive control in repair experiments since XP cells are known to be sensitive to 4NQO¹³ due to the defective repair of 4NQO-induced DNA base damage¹⁴.

Figure 1 compares the survival responses of the cell strains to killing by 4NQO. Both AT4BI and in particular AT2BE are abnormally sensitive to 4NQO, whereas AT3BI demonstrates a response similar to that of strains from two normal donors, GM 38 and GM 498. For example, when survival levels at 0.2 μ M 4NQO are compared with those of the normal GM 38 control strain, then AT2BE, AT4BI and AT3BI yield ratios of survival values of 3.2-, 1.7- and 1.0-fold respectively. Compared with ionizing radiation, chemical agents induce a relatively narrow spectrum of lesions in cellular DNA². Thus interstrain differences in the responses of AT cells to 4NQO and other radiomimetic chemicals^{2,15,16} probably reflect the different DNA repair pathways in which AT strains are malfunctional.

The principal types of DNA damage induced by the active cellular metabolite of 4NQO are one stable adduct with adenine and two stable and one unstable adduct with guanine^{14,17,18}. The

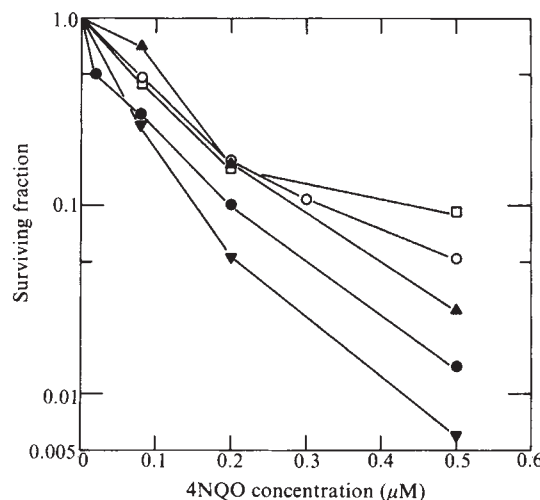


Fig. 1 Cell survival against 4NQO dose for normal and AT fibroblasts. Cells were collected from late logarithmic phase cultures and allowed to attach at 2×10^2 – 2×10^4 cells per 10-cm diameter plastic dish (Lux Scientific Corporation) containing growth medium at 37 °C for a period of 6 h. Attached cells were then washed with phosphate-buffered saline and incubated for 1 h at 37 °C with the indicated dilution of 4NQO in serum-free growth medium. Following the chemical treatment, the cultures were washed with growth medium and clonogenic potential was measured using the feeder layer technique previously described⁷. Data points represent the means of independent determinations (number in parentheses) for: ○, GM 38 [5; plating efficiency (PE) range, 7–21%]; □, GM 498 [1; PE, 6%]; ▲, AT2BE [3; PE range, 2–6%]; ●, AT3BI [2; PE, 2% and 5%]; ▼, AT4BI [4; PE range, 4–8%]. s.e. for each datum point was routinely $\leq 10\%$.

Table 1 General description and *in vitro* DNA repair characteristics of diploid fibroblast strains

Strain	Details of donor			Repair of ionizing radiation damage*		
	Diagnosis	Age (yr)	Sex	Repair replication	Removal of base damage	Strand-break rejoining
GM 38	Normal	9	F	+	+	+
GM 498	Normal	3	M	+	NT	+
AT2BE	AT	7	F	-	-	+
AT3BI	AT	4	M	-	-	+
AT4BI	AT	6	M	+	+	+
XP9RO	XP	9	M	+	NT	+

Monolayer stocks of each fibroblast strain (whose origin was human skin) were cultured in Ham's F12 medium supplemented with 15% (v/v) heat-inactivated fetal bovine serum, 1 mM glutamine, 100 U ml⁻¹ penicillin G and 100 µg ml⁻¹ streptomycin sulphate (growth medium). All culture procedures have been described previously⁷. Solutions of 4NQO (K & K Laboratories) were prepared immediately before use by dissolving the solid in 100% ethanol at room temperature and diluting to the appropriate concentration in pre-warmed serum-free growth medium.

* Details of DNA repair characteristics for the normal strains (our unpublished data), AT strains^{2,6,8} and the XP strain²⁵: +, proficient; -, deficient; NT, not tested.

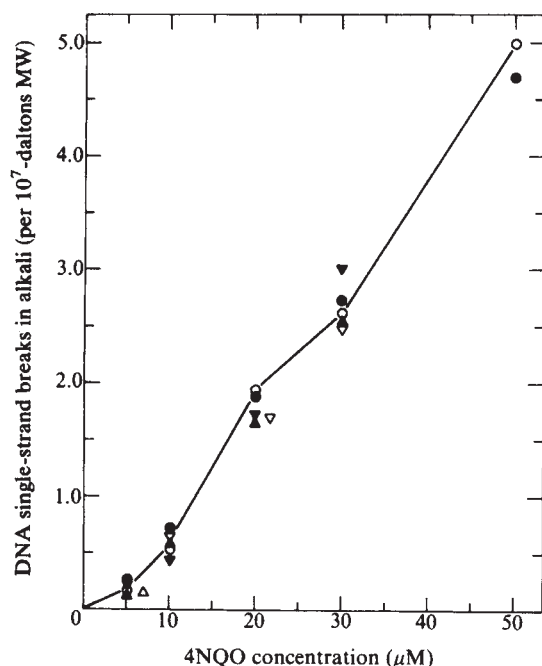


Fig. 2 4NQO dose-dependent induction of alkali-labile lesions in the DNA of normal, AT and XP fibroblasts. Cells were grown for ~48 h at 37 °C in medium containing either (*Me*-³H)thymidine (1 µCi ml⁻¹; stock specific activity, 6.7 Ci/mmol⁻¹; NEN Canada) or [*Me*-¹⁴C]thymidine (1 µCi ml⁻¹; 40.8 mCi mmol⁻¹; NEN). Tritium-labelled cultures (4×10^5 – 6×10^5 cells per 10-cm diameter dish) were exposed for 1 h to the appropriate concentrations of 4NQO as described in Fig. 1 legend. The ¹⁴C-labelled control cultures were handled in a similar manner but not exposed to 4NQO. The parallel cultures were lysed and DNAs from the untreated control (¹⁴C) and carcinogen-treated (³H) cells were co-extracted with 0.5 M Tris-HCl (pH 8.0)-saturated phenol and dialysed against 0.05 M Na₂PO₄-1 mM EDTA buffer (pH 7.0). Co-extracted, radioactively labelled DNAs (100 µl) were incubated with unlabelled, sonicated calf thymus DNA (40 µl; 1 µg ml⁻¹ stock; included to compete for any nonspecific nuclease activity) at 37 °C for 30 min followed by the addition of 1 M NaOH (100 µl). The DNAs were analysed by velocity sedimentation (40,000 r.p.m., 20 °C, 120 min in a Spinco SW-56 rotor driven by a Beckman LS-65 ultracentrifuge) in alkaline sucrose gradients (5–20%, w/v); 0.5 M NaCl, 0.2 M NaOH, 10 mM EDTA; pH adjusted to 12.5). After gradient fractionation and liquid scintillation counting, computer analysis of the gradient profiles yielded the number of strand breaks in alkali in the 4NQO-treated ³H-DNA compared to the control ¹⁴C-DNA. Data points represent arithmetic means of at least two separate determinations. ○, GM 38 (solid line); ▽, XP9RO (5 and 20 µM points offset for clarity); ▼, AT2BE, ▲, AT3BI; ●, AT4BI. s.e. for each datum point was ≤10%. MW, Molecular weight.

unstable guanine adduct appears to give rise to a DNA single-strand break in alkaline conditions^{17,19,20}. The unstable 4NQO-guanine adducts are removed by a process(es) which may involve an ionizing radiation-like repair pathway(s)⁹ and these defects are therefore candidates for the lethal lesions in the radiosensitive strains AT2BE and AT4BI. There is evidence in both bacterial²¹ and mammalian cells^{9,14} that the stable purine adducts are repaired by a UV-light-like excision repair pathway which leads to extensive DNA repair synthesis.

Figure 2 shows the 4NQO dose-dependent induction of single-strand breaks, detected in alkaline conditions, for several cell strains. The three AT strains examined all show similar levels of DNA damage to those of normal (GM 38) and XP (XP9RO) strains, despite their different sensitivities to the carcinogen.

Since the survival responses of AT strains to 4NQO could not be explained by differential susceptibility to DNA damage, we examined the repair kinetics of the alkali-labile lesions for the five strains (Fig. 3). In the normal strain (GM 38) and the AT strain of normal 4NQO sensitivity (AT3BI), fewer than 10% of the initial number of lesions remain 6 h after 4NQO treatment. Analysis of samples at incubation times longer than 6 h was sometimes complicated by anomalous sedimentation characteristics of the DNA preparations. For the two AT strains that

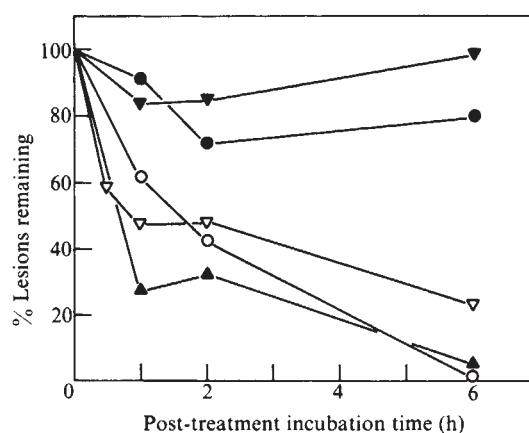


Fig. 3 Disappearance of 4NQO-induced alkali-labile lesions from the DNA of normal, AT and XP fibroblasts. The method is similar to that used to follow the repair of radiogenic single-strand breaks⁸, and the protocol is detailed in Fig. 2 legend. Post-treatment incubation was in growth medium at 37 °C. Data points represent arithmetic means of multiple determinations indicated in parentheses: ○, GM 38 (6); ▼, AT2BE (4); ●, AT4BI (4); ▽, XP9RO (3); ▲, AT3BI (2). s.e. for each datum point was ≤15%.

are hypersensitive to 4NQO (AT2BE and AT4BI), more than 75% of the initial number of lesions remain 6 h after chemical treatment. This repair defect is also observed at lower 4NQO concentrations (data not shown). The XP strain displays an essentially normal rate of disappearance of this type of lesion. Thus, the cell survival responses of the three AT strains correlate with their ability to remove the carcinogen-induced alkali-labile DNA lesions.

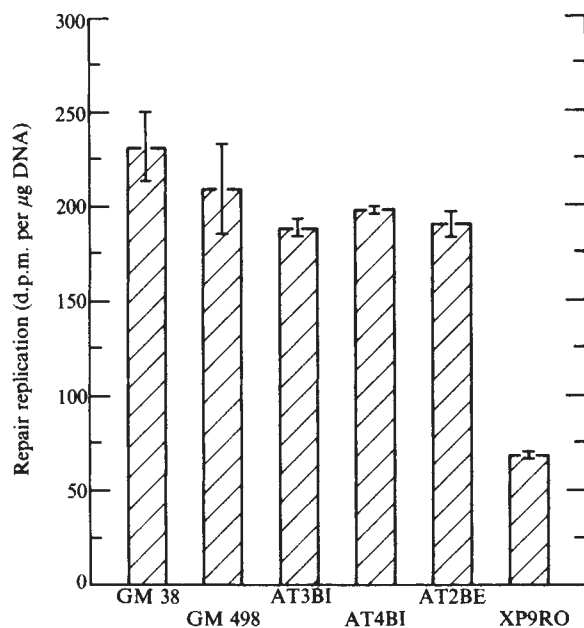


Fig. 4 DNA repair replication in normal, AT and XP fibroblasts exposed to 4NQO. The technique used was essentially that described for the measurement of γ -ray-induced DNA repair replication⁸. Repair replication is a measure of the incorporation of tritiated thymidine into non-replicating DNA during damage site repair. Growth medium was supplemented with 10% (v/v) dialysed calf serum rather than fetal bovine serum. Before 4NQO treatment, cultures (1×10^6 – 1.5×10^6 cells per 15-cm diameter dish) were incubated for 2 h in growth medium containing 6.5 μ M 5-bromodeoxyuridine (BUdR) and 1 μ M 5-fluorodeoxyuridine (FUDR). After exposure for 1 h to 30 μ M 4NQO, repair was followed in the presence of 6.5 μ M BUdR, 1 μ M FUDR, 1 mM hydroxyurea and 10 μ Ci ml⁻¹ [Me -³H]thymidine (stock specific activity, 41 Ci mmol⁻¹; NEN) for 2 h at 37°C. The labelling medium was then removed, and the cells were incubated for a further 1 h in medium containing 6.5 μ M BUdR and 1 μ M FUDR. DNA was then isolated for density gradient centrifugation by lysing the collected cells and extracting in chloroform-isoamyl alcohol. Neutral NaI gradients were prepared (6.2 ml containing 0.22 mM Na₂SO₃, 10–20 μ g extracted human DNA, 0.1 mg ethidium bromide, 4.69 g NaI, adjusted to a final density of 1.54 g cm⁻³) and centrifuged (33,000 r.p.m. at 30°C; ≥ 60 h in a Spinco Type 40 rotor). The gradients were each fractionated into 18 tubes for the estimation of DNA concentration in a spectrofluorometer (Mk 1; Farrand Optical Co.). One rebanding of 'light' parental DNA was carried out to reduce contamination by 'heavy' DNA arising from semiconservative *de novo* DNA synthesis. Rebanding involved: (1) pooling six fractions containing light DNA per gradient; (2) dialysis overnight against 10 mM Tris-0.15 M NaCl-10 mM EDTA (pH 7.5) buffer; (3) preparation; and (4) centrifugation of NaI gradients as above. The rebanded samples were again fractionated and DNA concentration measured. Gradient fractions were transferred into ice-cold 10% trichloroacetic acid and 10 mM Na₂P₂O₄ and the precipitated, radioactive DNA collected on Whatman GF/C filter papers, and counted by liquid scintillation in toluene-Scintiprep 2 (Fisher Scientific). Histograms represent the arithmetic mean (background corrected) of two determinations for each designated strain with the range denoted by error bars.

Repair replication (a measure of repair synthesis) was measured for 2 h following exposure of cultures to 30 μ M 4NQO. The results shown in Fig. 4 indicate that the technique clearly detects the defect in repair synthesis expressed by an XP cell strain^{22–24}. However, there was no significant difference between the levels of repair in the two normal controls and the levels in the three AT cell strains. Since the expression of repair synthesis is considered to reflect largely the repair, by UV-like pathways, of the stable 4NQO-purine adducts¹⁴, we conclude that such lesions are repaired with normal efficiency in AT strains. This view, that UV-like repair pathways are not defective in AT strains, is consistent with observations that far UV-induced repair synthesis is not reduced in AT strains^{8,16} compared with normal cells.

As a DNA damaging chemical, 4NQO shows potential as a screening agent for DNA repair deficiencies in human cells. Its usefulness is facilitated in part by: (1) its dual UV-mimetic and radiomimetic properties, (2) its direct relevancy to carcinogenesis-related studies, and (3) the defined range of induced DNA lesions whose repair can be independently monitored.

Our data point to the presence in some AT cell strains of a defect in the repair of the unstable class of 4NQO-guanine adducts. We propose that the repair defect lies in the initial enzymatic release of the base adduct. Our results provide the first evidence of the nature of the putative DNA repair defect in AT4BI. Genetic heterogeneity in the repair abilities of AT strains following γ -ray or 4NQO treatment may be explained on the basis of deficiencies in either a pathway which handles γ -ray damage (for example, in AT3BI) or a pathway which handles γ -ray and 4NQO damage (for example, in AT2BE and AT4BI). Future research into the cellular and DNA repair properties of AT cells should provide a greater insight into the role of host and environmental factors in the process of neoplastic transformation and neurological degeneration.

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GM 38 and GM 498 were from the Human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, New Jersey; AT2BE (CRL 1343) was from the American Type Culture Collection; AT3BI was supplied by Dr A. R. Lehmann, AT4BI by Dr A. M. R. Taylor, and XP9RO (complementation group C¹⁶) was a gift from Dr D. Bootsma. Cell culture supplies were from Microbiological Associates.

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Dimeric tRNA precursors in yeast

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Two DNA fragments, each containing a tRNA^{Arg} and a tRNA^{Asp} gene in close conjunction, have been isolated from different genomic regions of *Saccharomyces cerevisiae*¹. Nucleotide sequence analysis of the gene regions revealed that in both fragments the tRNA^{Arg} coding region is located 5'-proximal to the tRNA^{Asp} coding region. They are separated by an identical spacer of 10 nucleotides. Although the 5'-flanking sequences are different in the two plasmids, some similarities are observed. To test the mode of expression of this gene configuration, we transcribed the DNA fragments in a *Xenopus* oocyte nuclear extract^{2,3}. Specific transcription of the yeast tRNA genes took place in an RNA precursor which comprised both tRNA species. We report here that the precursor RNA was processed to the mature-sized tRNA molecules, indicating the presence of an enzyme activity in the *Xenopus* nucleus capable of cutting a dimeric tRNA precursor. This is the first observation of a eukaryotic dimeric tRNA precursor.

The tRNA genes of several eukaryotes have recently been shown to be organized in a variety of ways. In some cases, such as *Drosophila* and *Xenopus*, multiple copies of the same gene or a number of different genes are clustered⁴⁻⁷. On the other hand, in nematode⁸, silkworm^{9,10} and yeast¹, tRNA genes generally seem to be scattered throughout the genome. Pulse-chase experiments of RNA in mammalian cells¹¹ and UV-light mapping studies of transcription units in yeast¹² have shown that dimeric or multimeric tRNA precursors are very unlikely to exist. In addition, *in vivo* and *in vitro* transcription studies in the *Xenopus* system have determined that eukaryotic tRNA genes are organized as single transcription units^{3,6-10,13-15}.

The recombinant pBR313 plasmids pJB18u and pJB19f contain *Hind*III fragments of *S. cerevisiae* DNA¹. Hybridization patterns of [5'-³²P]tRNA to Southern blots of different DNA fragments from these plasmids suggested that the coding sequences for tRNA^{Arg} and tRNA^{Asp} are located in adjacent

positions¹⁶ and that they may be co-transcribed. The plasmid pJB18u contains, in addition, a tRNA^{Tyr} gene about 1 kilobase upstream of the tRNA^{Arg} and tRNA^{Asp} genes¹⁶. The DNA around the tRNA^{Arg} and tRNA^{Asp} position has been sequenced in both plasmids (Fig. 1). Up to position -12 the 5' flanking sequences are almost identical, but further out differ considerably in the two plasmids. This confirms the results of restriction enzyme analysis that the *Hind*III fragments in pJB18u and pJB19f derive from unique sites in the *S. cerevisiae* genome. In both plasmids the tRNA^{Arg} coding sequence is 5'-proximal to the tRNA^{Asp} coding sequence. They are separated by an identical 10-nucleotide spacer in pJB19f and pJB18u. The two plasmids contain the same tRNA coding sequences which are identical to the known tRNA^{Arg} (ref. 17) and tRNA^{Asp} (ref. 18) sequences. As in most other eukaryotic tRNA genes, the 3'-flanking sequences in both clones contain an oligothymidylate sequence which is probably a transcription termination signal for RNA polymerase III^{9,10,13,19}.

The transcription of these tRNA genes was tested in a nuclear extract of *Xenopus* oocytes^{2,3}. The radioactive RNA was separated by polyacrylamide gel electrophoresis as shown in Fig. 2. Four major products designated RNA-1, RNA-2, RNA-3 and RNA-4 were observed. Using yeast 5S, yeast 5.8S and tRNA precursors of known length²⁰ as markers, the length of RNA-1 was determined to be 170 ± 10 nucleotides.

The first evidence that RNA-1 is a precursor to the tRNA-sized transcripts came from a pulse-chase experiment. After incubation with [α-³²P]CTP for 1 h, an excess of unlabelled CTP was added and the reaction allowed to proceed for another 2 h (Fig. 2). It is evident that RNA-1 and RNA-2 are less prominent in the chase experiment, indicating a precursor-product relationship to RNA-3 and RNA-4. This result was confirmed by reincubation of isolated radioactive RNA-1 in GV extract, which led to formation of RNA-3 and RNA-4 (not shown).

The nucleotide sequence of these RNA species was established by conventional methods²¹ and compared with the DNA sequence. The RNase T₁ fingerprint of RNA-1 displayed all the fragments expected for the two tRNAs (Fig. 3a, Table 1). In addition, the fragments CUUUG (fragment 4) and UUCU-UCCG (in fragment 2) were present indicating that the spacer sequence between the two tRNA coding regions is transcribed. The fact that the mature 5' end of the tRNA^{Arg} is not found in RNA-1 implicates additional nucleotides in the form of a leader sequence. RNA-4 contained all the RNase T₁ fragments of tRNA^{Asp} (Fig. 3c). Analysis of a separate [α-³²P]CTP-labelled RNA-4 revealed the mature ends to be pUCCG, which apparently co-migrates with CUUG (fragment 6), and CCA_{OH} (Table 1). Additional evidence that the 3' end contains the CCA_{OH} oligonucleotide derives from the fact that the preceding RNase T₁ fragment (AG) is labelled in this RNA (Table 1).

Fig. 1 Nucleotide sequences of the anticoding strand of the tRNA^{Arg}-tRNA^{Asp} genes from plasmids pJB18u and pJB19f. They are aligned according to the tRNA coding sequences (boxed). The chemical degradation method of Maxam and Gilbert²⁴ was used for DNA sequencing. The pJB18u sequence was determined from a *Taq*I site at position 55 in opposite directions for ~110 nucleotides. The elucidation of the pJB19f sequence of a *Cl*aI-*H*paI subfragment (440 base pairs) was done from the *Cl*aI (position -235), the *H*paI (position 199), the *H*aeIII (position -119) and the *S*au3AI (position 127) site. The identity of each nucleotide was determined at least twice.

		-230	-220	-210	-200	-190	-180	-170		
PJB 18U	5'	-----								
PJB 19F	5'	-----								
		-160	-150	-140	-130	-120	-110	-100	-90	
PJB 18U		-----								
PJB 19F		-----								
		-80	-70	-60	-50	-40	-30	-20	-10	
PJB 18U		-----								
PJB 19F		-----								
		AATTGGTGAAATCAAAGCATAGTTTACCTTTAATCCCAATCTCCACAAACA								
PJB 18U		CCGTGAAATCCGAAATTTAGTAGTAATTGAAAGCGTAATTAGGTTTTACTATAATAAAGTAGTAAACCTTCAACAAATA								
PJB 19F		CCGTGAAATCCGAAATTTAGTAGTAATTGAAAGCGTAATTAGGTTTTACTATAATAAAGTAGTAAACCTTCAACAAATA								
		1	ARG	10	20	30	40	50	60	70
PJB 18U		-----								
PJB 19F		-----								
		GTAGCTCGCGTGGCGTAATGGCAACGGCTCTGACTTCTAATCAGAAGATTATGGGTTGCAGCCCCATCGTGAGTGCTTTG								
PJB 18U		GTAGCTCGCGTGGCGTAATGGCAACGGCTCTGACTTCTAATCAGAAGATTATGGGTTGCAGCCCCATCGTGAGTGCTTTG								
PJB 19F		GTAGCTCGCGTGGCGTAATGGCAACGGCTCTGACTTCTAATCAGAAGATTATGGGTTGCAGCCCCATCGTGAGTGCTTTG								
		80	ASP	90	100	110	120	130	140	150
PJB 18U		-----								
PJB 19F		-----								
		TTTCTCCGTGATAGTTTAATGGTCAGAATGGCGCTTGTGCGGTGCCAGATCGGGGTTCAATTCCCGCTCGGGGAGCTTT								
PJB 18U		TTTCTCCGTGATAGTTTAATGGTCAGAATGGCGCTTGTGCGGTGCCAGATCGGGGTTCAATTCCCGCTCGGGGAGCTTT								
PJB 19F		TTTCTCCGTGATAGTTTAATGGTCAGAATGGCGCTTGTGCGGTGCCAGATCGGGGTTCAATTCCCGCTCGGGGAGCTTT								
		160	170	180	190	200	-----3'			
PJB 18U		TTTTT-----								
PJB 19F		TTTTTGGCTACTCCTGTAGTTATCTTCATTAAATGCTTTGTTAACTTT								

Table 1 Analysis of RNase T₁ fragments

No.	Fragment	RNA-1		RNA-3		RNA-4		
		Labelled with [α - ³² P]GTP†	Deduced‡	Labelled with [α - ³² P]GTP*†	Deduced‡	Labelled with [α - ³² P]GTP†	Deduced‡	Deduced‡
1	ACUUCUAAUCAG UUCAAUUCGCCG	AG, C	ACUUCUAAUCAG UUCAAUUCGCCG	AG	AAU, AC, U	ACUUCUAAUCAG	C	UUCAAUUCGCCG
2	UUUAAUG UUUCUUCCG	AAU, G, C	UUUAAUG UUUCUUCCG	—	—	AAU, G	—	UUUAAUGG
3	AUUAUG	AU, G	AUUAUGG	AU, G	—	AUUAUGG	—	—
4	CUUUG	U	CUUUG	—	—	—	—	—
5	UAAUG	AAU, G	UAAUGG	AAU, G	—	UAAUGG	—	—
6	UUCG, UCUG, CUUG	C, U	UUCG, UCUG, CUUG	C, U	G, U	UUCG, UCUG	U	CUUG
7	ACCCCAUCG	C	ACCCCAUCG	C	AC, AU, C	ACCCCAUCG	—	—
8	AAUG, AUAG	AAU, AG, G	AAUGG, AUAG	—	—	AAU, AG, G	—	AAUGG, AUAG
9	AUCG, UCAG	AG, G, C	AUCGG, UCAG	—	—	AG, G, C	AU, U	AUCGG, UCAG
10	AU	AU	AUG§	AU	—	AUG§	—	—
11	CUCG	C	CUCG	C	G, U	CUCGC	—	—
12	UAG	AG	UAG	—	—	—	—	—
13	UCG	C	UCG	—	—	C	G, U	UCGC
14	UG	G, U	UG, UGG	G, U	G	UGC, UGG	U	UGC
15	CAACG	AAC	CAACG	AAC	AAC, G	CAACGC	—	—
16	CCAG	AG	CCAG	—	—	AG	C	CCAG
17	AAG	AAG	AAG	AAG	—	AAG	—	—
18	AG	AG	CAG§	AG	—	CAG§	—	—
19	AG	AG	AG	AG	—	AG	AG	AGC
20	CG	C	CG, CGG	C	—	CG	G, C	CGG
21	G	G	G	G	G	G	G	G
22	CCA _{OH}	—	—	C	CCA _{OH}	—	C	CCA _{OH}

* From DNA sequence.

† Digested with RNase A.

‡ Bases in italics were deduced from nearest neighbour analysis.

§ These are overdigestion products of fragments 1 and 3.

although its nearest 3' neighbour is A in the original transcript. An RNase T₁ fingerprint of RNA-3 (Fig. 3d) revealed all the oligonucleotides for tRNA^{Arg} (Table 1) including the mature 5' end (pGp). Furthermore, the CCA_{OH} oligonucleotide was found in a [α -³²P]CTP-labelled RNA (Table 1). Preliminary fingerprint analysis indicates that RNA-2 contains a mixture of the tRNA species and probably represents the cleavage product from the dimeric precursor. However, other events, such as transcription termination in the intergenic spacer region, cannot be ruled out.

Thus, the sequence analysis of the transcription products confirms the notion that the two RNA sequences are contained in a large transcription product including the spacer and probably flanking sequences. We suggest that dimeric tRNA precursors do exist in yeast, although they are not abundant. The dimeric transcript is processed in the nuclear extract to yield the

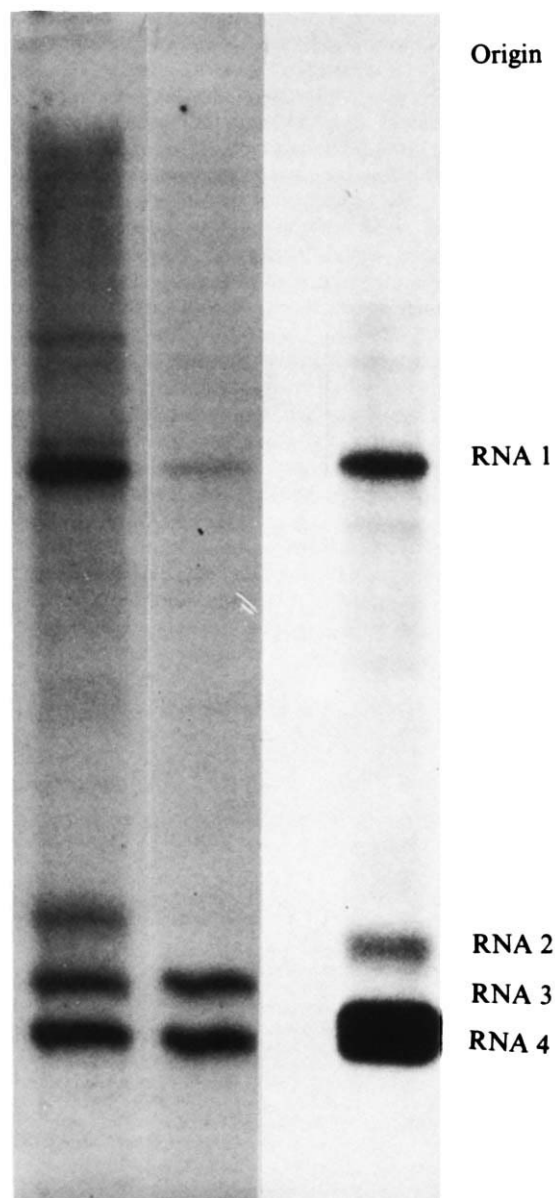
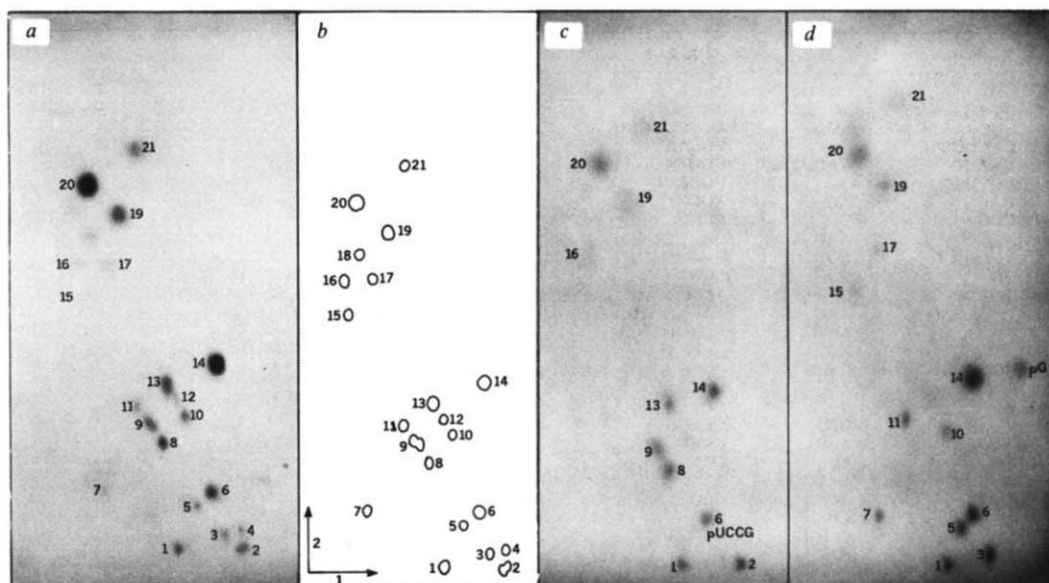


Fig. 2 Transcription and processing in *Xenopus* nuclear extracts. Autoradiograms of RNA transcribed from plasmids pJB18u and pJB19f in a variety of conditions separated on 10% polyacrylamide-4 M urea gels. The transcription reactions (40 μ l) contained 30- μ l GV extracts, 1 μ g DNA, ATP, UTP, and GTP (0.03 mM each) and [α -³²P]CTP (0.01 mM, 40 Ci mmol⁻¹) in NH₄Cl (70 mM), MgCl₂ (7 mM), EDTA (0.1 mM), HEPES (10 mM, pH 7.4), dithiothreitol (2.5 mM) and 1% polyvinylpyrrolidone. After incubation at 22 °C for the specified length of time, the RNA was extracted¹⁴ and separated by electrophoresis. Lane 1, pJB19f transcription for 1 h; lane 2, after 1 h in pJB19f transcription, reaction (lane 1) was supplemented with cold CTP and the incubation continued for a further 2 h; lane 3, pJB18u transcription for 1 h and separated on a different gel without urea.

Fig. 3 RNase T₁ fingerprints of: *a*, RNA-1; *c*, RNA-4; *d*, RNA-3 of pJB19f DNA transcripts labelled with [α -³²P]GTP. *b* is a diagram of a fingerprint of RNA-1. The numbering corresponds to that in Table 1. Standard RNA fingerprint separations were by electrophoresis on cellulose acetate at pH 3.5 (I) and on DEAE-cellulose at pH 1.7 (II).



mature-sized tRNA molecules. A number of nucleotide modifications are also found in these RNA species. Analysis of C-labelled RNA-3 and RNA-4 revealed ψ and m¹A residues present in the position expected from the sequence of the mature tRNAs^{17,18}. It is not known whether a specific endonuclease activity is required to cleave the dimeric precursor or whether the nucleases processing monomeric transcripts can perform this function. The analysis of a different dimeric tRNA precursor in *Schizosaccharomyces pombe* indicated an endonuclease activity to cleave at the 5' end of the second tRNA²⁰. This activity, together with the activities involved in the processing of the 3'-flanking sequences are present in the nuclear extract^{9,10,17}. Therefore, these processing steps are likely to occur in the nucleus, which has also been shown by *in vivo* experiments²².

As these experiments involved a heterologous system, the results need to be confirmed in a yeast transcription system or by Northern hybridization analysis²³ of yeast RNA. Our results agree with the existence of tRNA-related yeast RNA species of greater than 5S RNA size, which have been shown in short *in vivo* pulse-labelling experiments (R. M. Bock, personal communication).

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The protein-coding sequence of the bovine ACTH- β -LPH precursor gene is split near the signal peptide region

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The pituitary hormones corticotropin (ACTH) and β -lipotropin (β -LPH) are formed from a large common precursor¹⁻⁸. Recently, we have elucidated the whole primary structure of the bovine ACTH- β -LPH precursor (designated alternatively as preproopiomelanocortin) by determining the nucleotide sequence of cloned DNA complementary to the mRNA coding for the precursor protein⁹. The amino acid sequence assigned has disclosed a characteristic repetitive structure of the ACTH- β -LPH precursor. The repetitive units of the precursor protein each contain a melanotropin (MSH) sequence (α -, β - or γ -MSH) as well as other peptide components such as β -endorphin and corticotropin-like intermediate lobe peptide (CLIP). The repetitive units as well as their peptide components are each bounded by paired basic amino acid residues, which apparently represent the sites of proteolytic processing. Several studies¹⁰ have confirmed the translational initiation site and protein structure assigned (see also ref. 11 and refs therein). In view of the recent knowledge about the organization of eukaryotic genes (see refs 12, 13 for reviews), it would be of particular interest to investigate the relationship between the repetitive structure of the ACTH- β -LPH precursor containing different functional components and the arrangement of the protein-coding sequence in its gene. We have now isolated and characterized bovine genomic DNA fragments encoding this precursor protein and have demonstrated that the protein sequence is encoded by two non-consecutive DNA segments. An intron (intervening sequence) of approximately 2.2 kilobase pairs separates the smaller exon (mRNA-coding sequence), which contains the gene sequence encoding the signal peptide, from the larger exon, which contains the gene sequence for most of the protein structure, including the known biologically active component peptides.

Calf thymus DNA was digested with various restriction endonucleases or their combinations and then subjected to agarose gel electrophoresis. DNA fragments containing an ACTH- β -LPH precursor gene sequence were identified by the Southern blotting method¹⁴ with the cloned cDNA (*Pst*I-excised insert of plasmid pSNAC20⁸) as a probe. Three hybridization-positive *Eco*RI fragments of 0.9, 1.5 and 3.5 kilobase pairs were detected. For further characterization of these fragments, we used two additional probes produced by *Eco*RI digestion of the cDNA insert, which has a single *Eco*RI site corresponding to the carboxy-terminal amino acid residues of ACTH⁹. The cDNA segment representing the 5' side of the mRNA (AL_L) hybridized exclusively to the 0.9 and 3.5-kilobase pair fragments, whereas that representing the 3' side of the mRNA (AL_R) hybridized exclusively to the 1.5-kilobase pair fragment (for the AL_L and AL_R segments, see Fig. 1e). Further restriction patterns obtained with various endonucleases and their combinations enabled us to construct a restriction map of the ACTH- β -LPH precursor gene as shown in Fig. 1a. The 0.9- and the 1.5-kilobase pair fragments are adjacent to each other and are located on the left and the right side, respectively, of the unique

*Eco*RI site present within the protein-coding sequence. The 3.5-kilobase pair fragment is positioned to the left of the 0.9-kilobase pair fragment.

To characterize the structural organization of the ACTH- β -LPH precursor gene, we isolated DNA clones carrying each of the three *Eco*RI fragments (for the cloning procedure, see Fig. 1 legend). Comparative restriction mapping with the Southern blotting technique confirmed that the cloned DNA fragments retained the sequence organization found in the cellular DNA. In addition, hybridization-positive calf thymus DNA fragments prepared by partial *Eco*RI digestion yielded DNA clones carrying a 5.9-kilobase pair insert which contains the 3.5-, 0.9- and 1.5-kilobase pair fragments oriented consecutively in this order.

Figure 1c and d compare the restriction patterns of the cloned 0.9- and 1.5-kilobase pair fragments with those of the cDNA segments AL_L and AL_R, respectively. There was a clear one-to-one correspondence between some of the restriction fragments derived from the 1.5-kilobase pair fragment and all restriction fragments derived from the AL_R segment, except the fragment that contains the site corresponding to the 3' end (poly(A) addition site) of the mRNA (Fig. 1d, e). A one-to-one

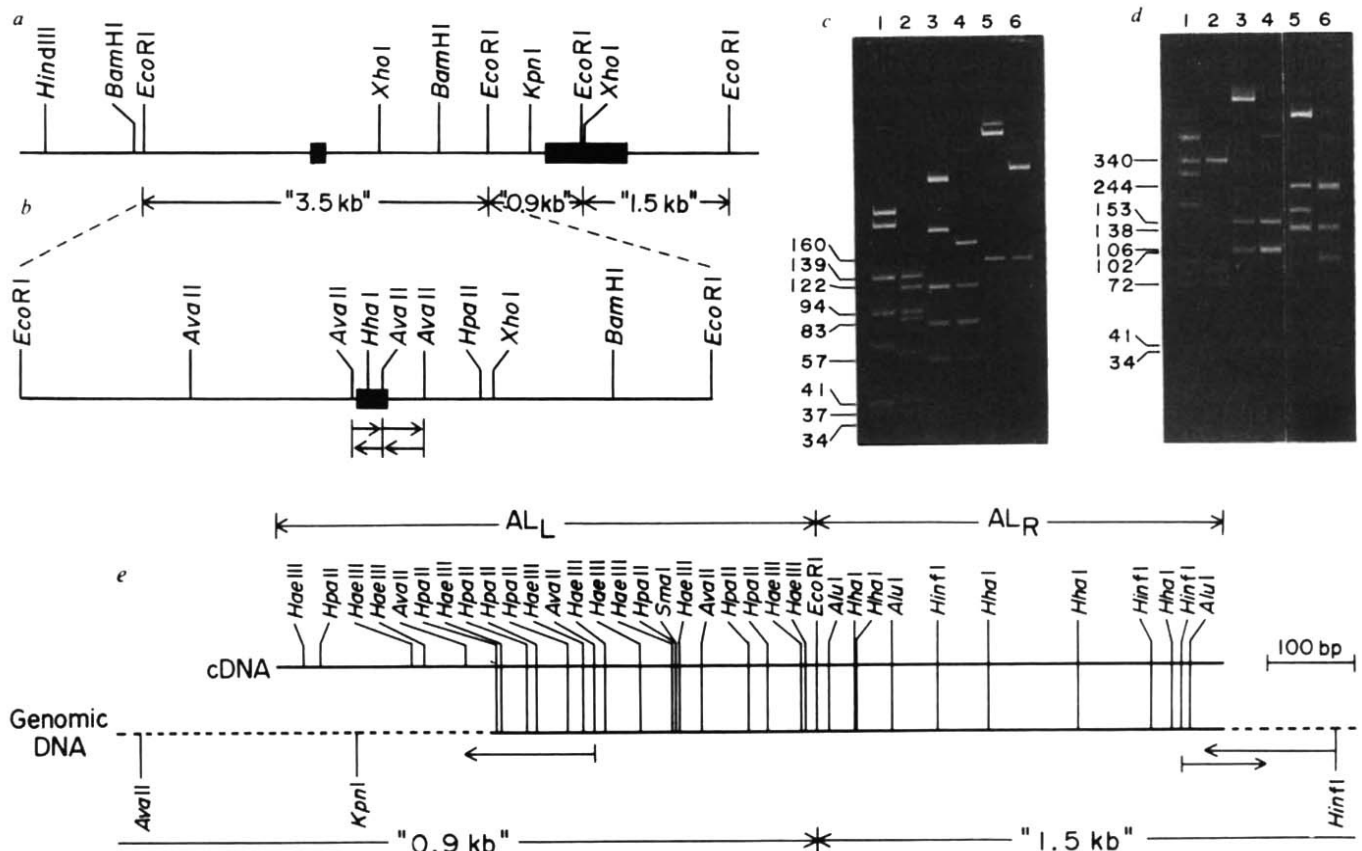


Fig. 1 Restriction mapping of the ACTH- β -LPH precursor gene in calf thymus DNA (a) and in the cloned *Eco*RI fragments of 3.5 kilobase pairs (b), 0.9 kilobase pairs (c, e) and 1.5 kilobase pairs (d, e). In a, b and e, the direction of transcription is from left to right. For reference, the locations of exons in a and b are indicated by closed boxes. Calf thymus DNA was isolated as described in ref. 21. In a, b and e, samples of cellular DNA digested to completion with the indicated enzymes or their combinations were electrophoresed on 0.7% agarose gels²¹ and transferred to nitrocellulose filters¹⁴. The probes used (see the text) were labelled with ³²P by nick translation²². Pre-hybridization, hybridization and filter washing were carried out at 65°C (ref. 23). In b, c and d, DNA clones were obtained as follows. Calf thymus DNA fractions containing the *Eco*RI fragments of 3.5, 0.9 or 1.5 kilobase pairs were isolated by preparative agarose gel electrophoresis²¹ and ligated to the purified *Eco*RI arms of bacteriophage λ gtWES- λ B (ref. 24). The recombinant DNA was packaged *in vitro* into viable phage²⁵, which were screened by hybridization with ³²P-labelled cDNA²⁶. Each *Eco*RI fragment was isolated from these recombinant phage and subcloned in pBR322 (ref. 27). In b, samples of the 3.5-kilobase pair ("3.5 kb") fragment digested with the indicated enzymes or their combinations were electrophoresed on 5% polyacrylamide gels⁹ or on 0.7% agarose gels²¹ and were stained with 0.1% ethidium bromide. The *Ava*II sites were located by partial digestion of the *Eco*RI-*Hpa*II fragment on the left side; the *Hpa*II end was 5'-³²P-labelled. The horizontal arrows indicate the direction and extent of sequence determinations. In c, samples of the 0.9-kilobase pair ("0.9 kb") fragment (lanes 1, 3, 5) and the AL_L segment (lanes 2, 4, 6) were digested with *Hae*III (lanes 1, 2), *Hpa*II (lanes 3, 4) or *Sma*I (lanes 5, 6). The resulting restriction fragments were electrophoresed on 5% polyacrylamide gel⁹ and stained as described above. Restriction fragments common between the cloned genomic DNA and cDNA are indicated on the left side of the electropherogram by their sizes in base pairs (bp). Faint bands observed in the upper region of the gel are due to incomplete digestion of the DNA. In d, samples of the 1.5-kilobase pair ("1.5 kb") fragment (lanes 1, 3, 5) and the AL_R segment (lanes 2, 4, 6) were digested with *Alu*I (lanes 1, 2), *Hha*I (lanes 3, 4) or *Hinf*I (lanes 5, 6). Other details were as described for c. In lane 4, the 3'-end-containing fragment was not separated from the fragments of 106 and 102 base pairs. In e, the restriction maps of the 0.9- and 1.5-kilobase pair fragments have been constructed on the basis of the data in c and d and the results of *Ava*II digestion. The dashed lines represent the regions where no correspondence between the restriction pattern of the cloned genomic DNA fragments and that of the cDNA was observed; in these regions, only the restriction sites used for DNA sequencing are shown. The horizontal arrows just below the line representing the genomic DNA indicate the direction and extent of sequence determinations. The portions of the AL_L and AL_R segments that correspond to the poly(dG)-poly(dC) tails and the poly(A) tail are not shown.

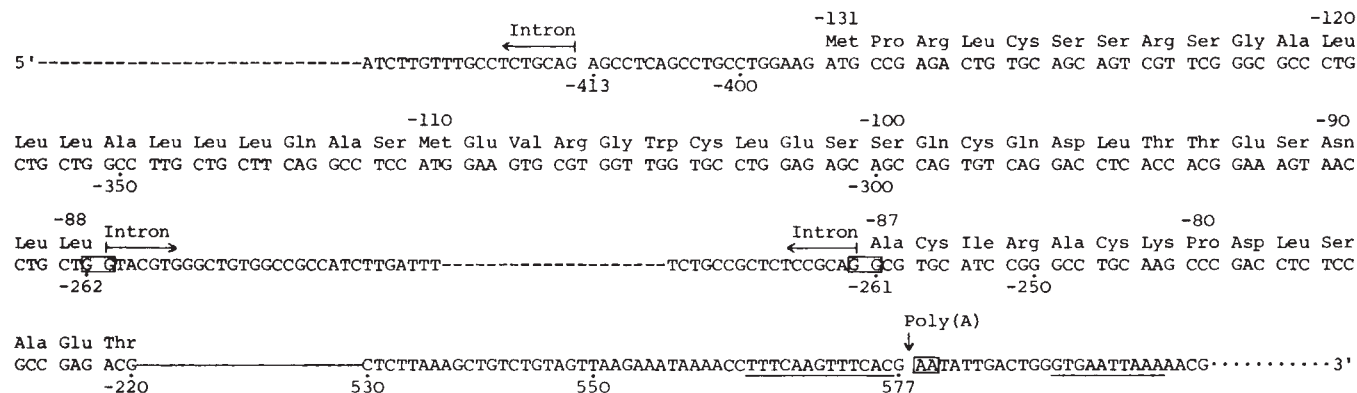


Fig. 2 Nucleotide sequence of the exon and its surrounding regions in the 3.5-kilobase pair fragment, the exon-intron junction in the 0.9-kilobase pair fragment and the region corresponding to the 3' end of the mRNA in the 1.5-kilobase pair fragment. 5'-End labelling of restriction fragments and DNA sequence analysis were carried out by the procedure of Maxam and Gilbert²⁸. The sequencing strategy was as follows: for the 3.5-kilobase pair fragment, the two *Ava*II fragments containing the exon (see Fig. 1b) were labelled and denatured, and both strands of each *Ava*II fragment were sequenced (sequence between the two dashed lines); for the 0.9-kilobase pair fragment, the *Ava*II fragment containing the exon-intron junction was labelled and cleaved with *Kpn*I (see Fig. 1e), and the *Ava*II-*Kpn*I fragment containing the exon-intron junction was sequenced (sequence between the second dashed line and solid line); for the 1.5-kilobase pair fragment, the 3'-end-containing *Hin*II fragment (see Fig. 1e) was labelled and denatured, and both strands were sequenced (sequence between the solid line and dotted line). The nucleotide sequence of the message strand is shown. Amino acid residues are numbered as in ref. 9. The numbers of nucleotide residues are those of the corresponding residues in the cDNA⁹. The solid line represents the exon sequence encoding the majority of amino acid residues and the 3'-noncoding segment of the mRNA. The dashed lines represent intron sequences, and the dotted line the 3'-flanking sequence. The exon-intron junctions indicated are positioned according to the GT/AG rule¹⁶. The redundant nucleotide residues at the exon-intron junctions and the residues corresponding to the ambiguous 3' terminus of the mRNA are boxed. The nucleotide residues representing an inverted repeat are underlined.

correspondence was similarly observed between some of the restriction fragments derived from the 0.9-kilobase pair fragment and all restriction fragments derived from the AL_L segment that are located less than 0.37 kilobase pairs upstream from the unique *Eco*RI site (Fig. 1c, e). In contrast, the restriction fragments positioned further upstream in the AL_L segment were completely displaced by unidentical restriction fragments derived from the 0.9-kilobase pair fragment. In agreement with these results of restriction mapping, nucleotide sequence analysis located the 3' end of the mRNA in the expected region of the 1.5-kilobase pair fragment, and an exon-intron boundary 373–374 nucleotide residues upstream from the right end of the 0.9-kilobase pair fragment (Fig. 2). We conclude, therefore, that one of the exons of the ACTH-β-LPH precursor gene is composed of 838–841 base pairs, which represent a major portion of the protein-coding sequence, specifying 221 amino acids from the alanine residue located 87 residues before the amino terminus of ACTH through the carboxyl terminus of the precursor protein, and the 3'-noncoding sequence of the mRNA. The site corresponding to the 3' terminus of the mRNA cannot be uniquely defined because the first two residues of the poly(A) tail could be transcribed from the genomic DNA. The 10 nucleotide residues immediately preceding the poly(A) tail have 7 residues in common with the model sequence TTTTCACTGC proposed by Benoist *et al.*¹⁵. Note also the presence of an inverted repeat (a region of two-fold rotational symmetry) surrounding the 3' end of the mRNA.

On the basis of restriction mapping of the cloned 3.5-kilobase pair fragment (Fig. 1b), a possible region containing the protein-coding sequence was located between 1.6 and 1.8 kilobase pairs upstream from the right end of this fragment. In fact, nucleotide sequence analysis of this region demonstrated a 156-base-pair sequence that corresponded precisely to the mRNA sequence starting 23 nucleotide residues upstream from the translational initiation codon (Fig. 2). Because the 3'-border sequence of this exon and the 5'-border sequence of the exon present in the 0.9-kilobase pair fragment form a consecutive sequence on the mature mRNA, we conclude that these two exons are separated by an intron of approximately 2.2 kilobase pairs on the genomic DNA. The border sequences of this intron exhibit a redundancy of two nucleotide residues. By appropriate selection of the splicing point, however, the intron follows the general rule proposed by Breathnach *et al.*¹⁶: it begins with a GT and ends with an AG dinucleotide.

Further mapping of the 3.5-kilobase pair fragment with restriction endonucleases known to cleave the region corresponding to the 5'-terminal sequence of the mRNA, in con-

junction with the failure of the resulting restriction fragments to hybridize to a cDNA sequence in this region, indicated that the mRNA-coding sequence upstream from that of the smaller exon is not included in the 3.5-kilobase pair fragment. Our attempts to identify a DNA fragment(s) containing the 5'-terminal mRNA structure have been unsuccessful. This small mRNA-coding segment may have been missed due to the relative instability of the hybrid formed during the Southern filter analysis.

Our results on the structural organization of the ACTH-β-LPH precursor gene are summarized in Fig. 3. Comparison of the restriction sites and partial DNA sequence of the cloned genomic DNA fragments with the known structure of the cDNA has demonstrated that the protein-coding sequence of this gene is split into two portions by an intron of approximately 2.2 kilobase pairs. This intron is located within the region corresponding to the peptide structure that exhibits some homology to the hormone calcitonin (L. J. Deftos, personal communication and refs 11, 17) and thus separates the gene sequence encoding the signal peptide¹⁸ from that encoding most of the protein sequence which includes different functional components corresponding to several biologically active peptides. A second intron is present within the region corresponding to the 5'-noncoding sequence of the mRNA as is the case for the ovalbumin gene¹⁹. Very recently, we have learned that Chang *et al.*²⁰ isolated a human genomic DNA segment containing a continuous sequence equivalent to the larger exon of the bovine ACTH-β-LPH precursor gene we identified.

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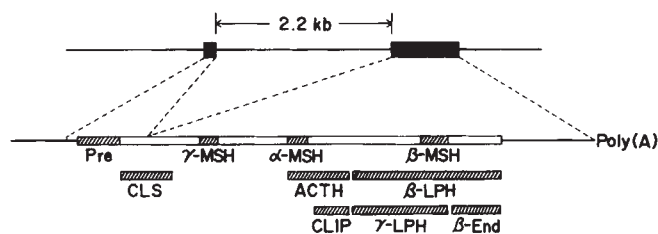


Fig. 3 Arrangement of the protein-coding regions in the bovine ACTH-β-LPH precursor gene. The exons are indicated by closed boxes on the upper line. For comparison, the topology of the mRNA is shown below. The shaded bars on and under the mRNA represent the regions coding for the component peptides of the ACTH-β-LPH precursor. Pre, signal peptide; CLS, calcitonin-like structure; β-End, β-endorphin.

Note added in proof: We have recently identified a third exon of 108 base pairs encoding the whole remaining 5'-terminal sequence of the mRNA by isolating additional bovine genomic DNA fragments and have thus confirmed the assigned position of the 3' end of the proximal intron, which has a length of approximately 4 kilobase pairs.

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Crystal structure analysis of a complete turn of B-DNA

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DNA is probably the most discussed and least observed of all biological macromolecules. Although its role in biology is a central one, with many examples such as operators and restriction sites where specific base sequences have control functions or interact with specific enzymes, the structures that DNA can adopt have been based until now only on sequence-averaged fibre diffraction patterns. Recent improvements in triester synthesis methods have made possible the preparation of sufficient homogeneous DNA of predetermined sequence for crystallization and X-ray structure analysis. We report here the first single-crystal structure analysis of more than a complete turn of right-handed B-DNA, with the self-complementary dodecamer sequence d(CpGpCpGpApApTpTpCpGpCpG) or CGCGAATTCGCG.

Two of the first crystal structure analyses of DNA prepared by the triester method, CGCGCG (ref. 1) and high-salt CGCG (ref. 2), produced variants of a totally unexpected left-handed zigzag or Z helix, of considerable structural interest but uncertain biological significance. The dodecamer sequence reported here was originally chosen to incorporate an *EcoRI* restriction endonuclease site, GAATTC, within ends related to the CGCG tetramer that was already being investigated in our laboratory. The discovery of the Z helix made this sequence potentially even more interesting as an example of two Z-compatible segments,

CGCG, bracketing a Z-incompatible AATT. We fully expected the structure to contain left-handed Z ends and a right-handed or melted-out centre. Instead, we found a right-handed B helix with slightly more than 10 base pairs per turn.

Crystals were grown from a pH 7.5 solution of DNA with a 2:1 molar excess of magnesium acetate, and one spermine hydrochloride molecule per eight base pairs, by vapour diffusion from 20 to 30% 2-methyl-2,4-pentanediol (MPD). Crystals 3 mm × 0.7 mm × 0.5 mm could be grown in a matter of weeks. These were broken up and used for heavy atom soaking trials; more perfect small crystals were used for data collection.

The dodecamer crystallizes in space group P2₁2₁2₁ with cell dimensions $a = 24.87 \text{ \AA}$, $b = 40.39 \text{ \AA}$ and $c = 66.20 \text{ \AA}$. An assumed crystal density of 1.5 g cm^{-3} leads to one double-stranded dodecamer per asymmetric unit in a cell 47% DNA by weight. Assuming 22 negative backbone phosphate charges per dodecamer, the above unit cell dimensions indicate that the crystals are 2.2 M in negative charge even if no account is taken of possible anions contributed by magnesium acetate. Because all of these must be counterbalanced by spermine or other cations, the dodecamer crystals are fully as 'high-salt' in local DNA environment as are CGCG (2.0 M) or CGCGCG (2.6 M, see ref. 2). If there had been a significant tendency for CGCGAATTCGCG to adopt a partial Z-helical conformation, it should have done so in the crystal. Instead, a right-handed B helix is formed, with not even an indication of local structure irregularity in the CGCG regions.

The distribution of X-ray intensities on survey precession photographs suggests a cross pattern centred on the c axis and shows a cluster of very strong reflections at $\sim 3.5 \text{ \AA}$ along the c axis, both implying that this is close to the direction of the DNA helix axis. Intensities remain strong in all directions out to $2.9 \text{ \AA}$, and then exhibit a rapid decline until essentially no data can be obtained beyond $1.9 \text{ \AA}$. Of the 5,691 possible reflections to $1.9 \text{ \AA}$ resolution, 2,818 were found to have an intensity greater than 2σ and were used in the analysis. Two isomorphous heavy atom derivatives were used: *cis*-dichlorodiamino platinum (II) obtained by diffusion, and a 3-Br derivative obtained by *de novo* synthesis of the dodecamer with 5-bromocytosine in the third position along each chain. The 1-Br derivative was crystallized but proved not to be isomorphous, and the 9-Br derivative was synthesized but not needed. Isomorphism in the *cis*-Pt derivative began to fail beyond $4\text{-\AA}$ resolution, but the 3-Br derivative remains isomorphous to $2.7 \text{ \AA}$.

The present report describes the partially refined structure obtained from multiple isomorphous replacement (MIR) analysis at $2.7 \text{ \AA}$ (mean figure of merit 57%), followed by Jack-Levitt refinement procedures³ using 2,725 2σ intensities between 8.0 and $1.9 \text{ \AA}$. The current residual error or R factor is 24.8% for a DNA molecule of 486 atoms and 9 initial water molecules. The structure of the DNA itself is essentially correct and is reported now because of its general interest. Refinement will continue with the addition of more solvent and spermine atoms, and some improvement in local nucleotide conformations.

A skeletal drawing of CGCGAATTCGCG is presented in Fig. 1, and a space-filling version from the same orientation in Fig. 2. The molecule is a Watson-Crick B helix with an average rise per residue of $3.4 \text{ \AA}$, and 10.1 base pairs per turn. This is somewhat less than the 10.4 ± 0.1 base pairs per turn as measured in solution by Wang⁴ or calculated by Levitt⁵ from energy considerations.

The deoxyribose rings in our structure have been fitted to electron density in the MIR map and then allowed to refine; no attempt has been made to impose a uniform C2'-endo or C3'-exo geometry on them. At this intermediate stage of refinement they are about equally distributed between C2'-endo and the O1'-endo configuration that is related by rocking about the glycosyl bond, but no conclusions about sugar puckering should be drawn until refinement is completed. No sequence-dependent variations in the uniform B helix have been seen, but these will be watched for as refinement continues.

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Fig. 1 Skeletal stereo drawing of CGCGAATTCGCG looking into the wide groove of the B double helix. Bases 1 and 24 are at the upper right and left in this view, and 12 and 13 are at the lower right and left. Note the uniform propeller twist in base pairs so as to maximize stacking contact with adjacent bases on the same strand, and the gentle curvature of the helix axis, concave to the right. For comments see text. Atoms in increasing order of size are C, N, O and P.

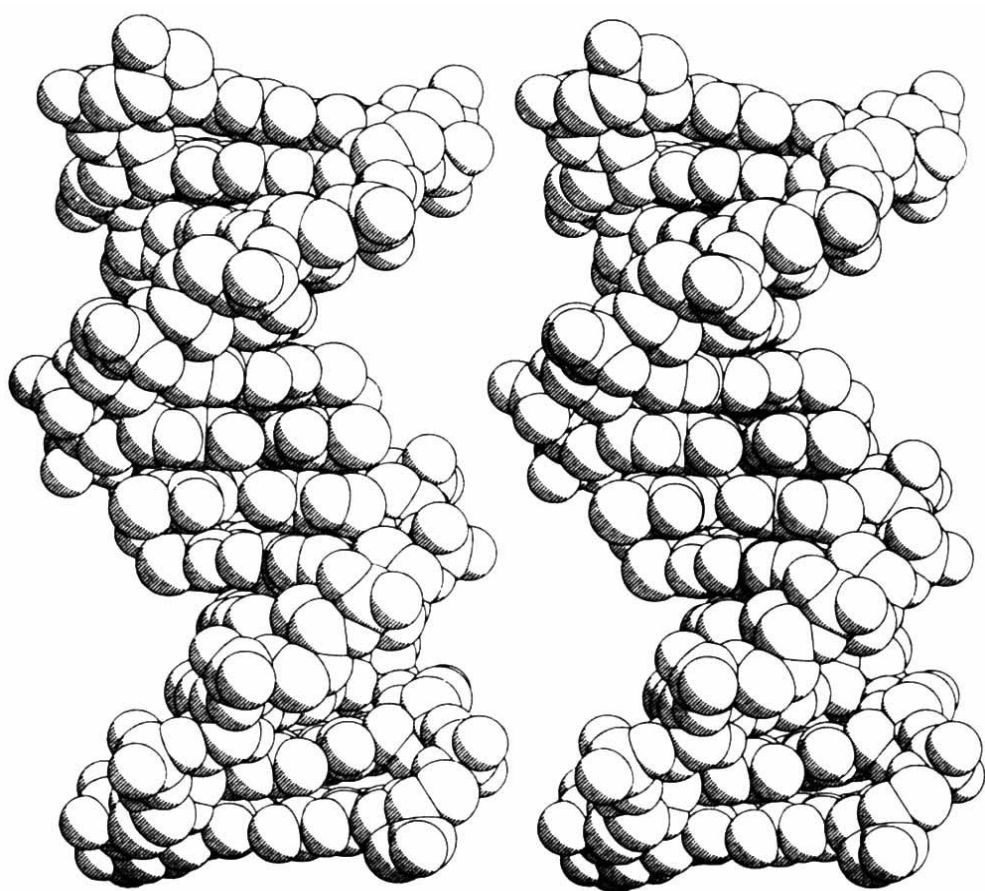
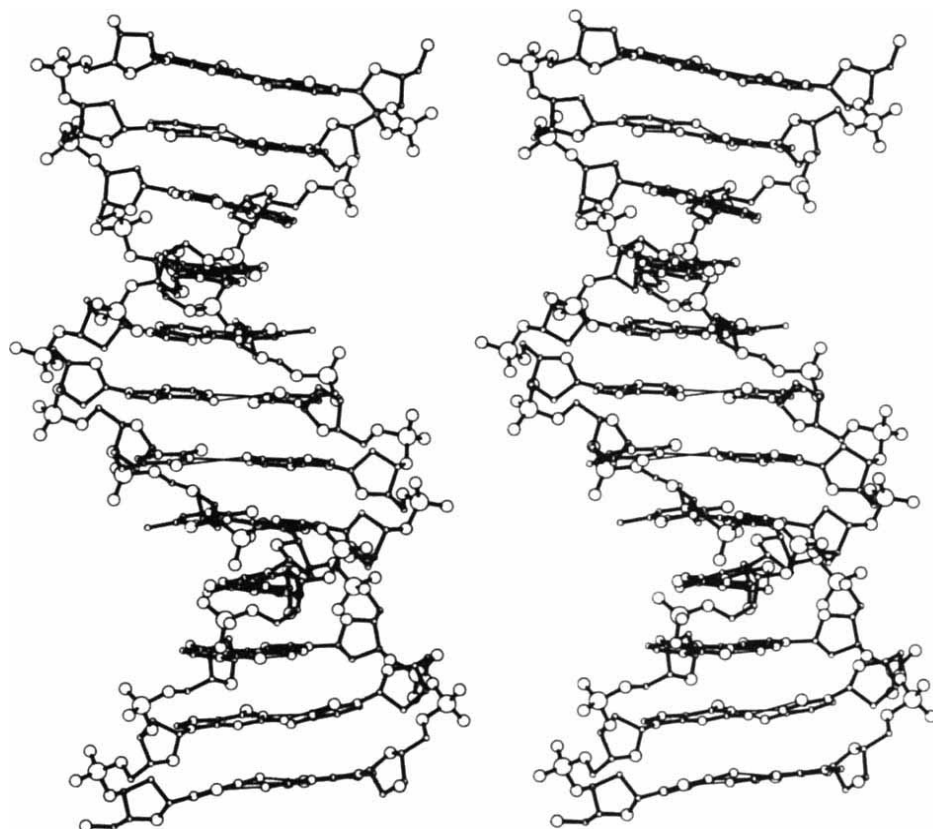
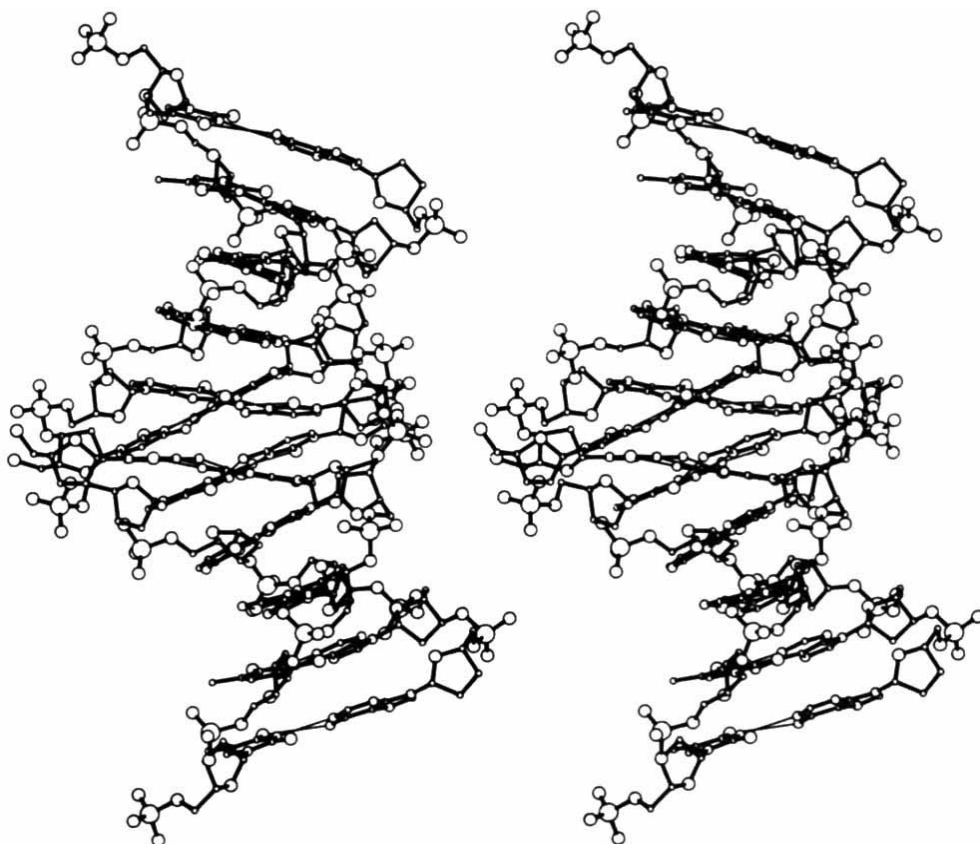


Fig. 2 Space-filling representation of CGCGAATTCGCG from the same viewpoint as Fig. 1. Atomic radii have been set at 85% of their true value for clarity. The advantage of propeller twist in maximizing adjacent base stacking can be seen in this packing drawing.

Fig. 3 Overlap of ends of molecules related by 2_1 axis along c (vertical). The upper half of one molecule (lower half not shown) sits in front of the lower half of another molecule (upper half omitted), with narrow grooves interlocked but with base planes tilted 35° to one another. The lower molecule shows bases 19–24 in front and 6–1 behind, and the upper molecule has bases 13–18 in front and 12–7 behind. The final base pair in each helix is packed parallel to, and in van der Waals contact with, the sugar–phosphate backbone of its neighbour. The narrow grooves at the ends of the molecules fit against one another like the palms of two cupped hands.



Even at this stage of the analysis, we can be confident about two interesting departures from classical B-helix geometry: each base pair has a propeller twist that increases the overlap between one base and its neighbours up and down the same chain, and the overall helix axis is slightly curved, concave to the right in Fig. 1. Levitt has proposed such a propeller twist in base pairs from energy calculations⁵, and has found it to be persistent and not especially sensitive to the particular choice of energy parameters for minimization. Moreover, Hogan *et al.*⁶ have found direct experimental evidence for propeller twist in solution from their transient electric dichroism measurements. This agreement

gives us confidence that the DNA conformation we observe in the crystal is very close to that which occurs in solution.

The axis of the dodecamer helix bends by 19° over 11 base steps, for a radius of curvature of 112 Å. Moreover, the plane of bending does not contain the internal 2-fold symmetry axis of the base sequence; it is roughly 90° away from it (Fig. 1). This non-use of sequence symmetry means that equivalent positions in the two chains experience different local stresses, and suggests that the curvature is induced by external influences rather than being inherent in the molecule. Little energy would be required to produce such curvature. If the molecule is regarded as an

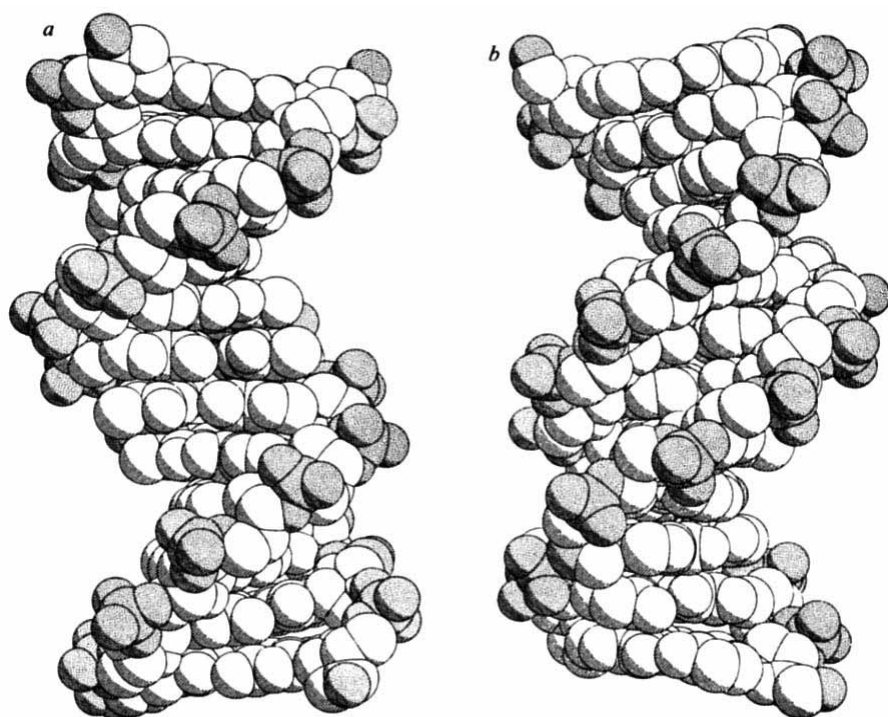


Fig. 4 Comparison of the wide groove (a) and narrow groove (b) in the B-DNA helix of CGCGAATTCGCG. Phosphate groups are shaded.

elastically deformable rod, the energy required for uniform bending is proportional to the rod length and inversely proportional to the square of the radius of curvature^{7,8}. The proportionality constant can be evaluated from the persistence length of DNA in solution, and lies between 85 and 170 kcal Å mol⁻¹ (refs 5, 8). This means that only 0.25–0.50 kcal of energy would be required per mol of dodecamer to produce the deformation seen in Fig. 1. As a comparison, the barrier to rotation about a single bond in ethane is nearly an order of magnitude greater, 3 kcal mol⁻¹. As crystal packing forces in proteins do frequently lead to different side-chain conformations in crystals where the asymmetric unit contains two molecules^{9,10}, such forces would be more than adequate to account for the observed curvature.

The probable origin of the crystal packing forces that produce the curvature is to be found in the association of neighbouring molecules up the 2₁ screw axis parallel to *c* as shown in Fig. 3. The molecules are not stacked on top of one another like cylindrical drums, instead they are staggered, with each molecule overlapping by three base pairs with its neighbours above and below. The overlap involves contact between minor grooves, with hydrogen bonds connecting ring N3 and amino N2 atoms in adjacent guanines from the two helices. (This pairing of guanines in the minor groove has been encountered in the structure of the complex of 9-ethylguanine and 1-methylcytosine¹¹, and has been proposed as a possible model for the recognition of guanine by asparagine or glutamine side chains of a protein (Fig. 3 of ref. 12).) Specifically, the N2 and N3 of guanine 14 in the upper molecule are hydrogen bonded to N3 and N2 of guanine 24 in the lower, and N2 and N3 of guanine 12 in the upper molecule are bonded to N3 and N2 of guanine 2 in the lower. However, base pairs in neighbouring molecules are sharply canted or tilted relative to one another by 35°, a tilt that brings the last base pair of one helix parallel to, and in packing contact with, the sugar–phosphate backbone of the other helix. The guanine N2...N3 hydrogen bonds are twisted but not broken, and the tilted structure is locked into place by one more hydrogen bond—from the 3'-terminal OH of the upper helix in fig. 3 to the N2 of guanine 22 in the lower helix. The extra stability provided by these five bonds is more than enough to produce a 19° curvature in the helix axis. This interlocking of helix termini, a function of the CGCG sequences with which the molecule begins and ends, is probably also responsible for the unusual ease and rapidity with which crystals of CGCGAATTCGCG can be grown, in comparison with other DNA molecules whose crystallization has been attempted in our laboratory and elsewhere.

The contrast between major and minor from grooves can be appreciated from Figure 4, in which the molecule is viewed from diametrically opposed directions. The ratio of widths of major and minor grooves along the helix axis is almost exactly 7:4, and the minor groove gives, in this space-filling model, the impression of being quite constricted.

The mode of binding of *cis*-dichlorodiamino platinum (II) to DNA is of considerable medical interest because the complex shows promise as a carcinostatic agent in cancer chemotherapy^{13,14}. The *cis*-Pt site in the dodecamer is in the vicinity of N7 and O6 of guanines 4 and 16 in the wide groove of the helix. As the *cis*-Pt complex is isomorphous with the native dodecamer only out to 4 Å resolution, it will be refined as an independent structural problem. The *trans*-Pt complex is even less isomorphous as judged by cell dimensions and intensity changes. All three refined structures will ultimately be reported, as a study of the mode of binding of platinum complexes to DNA.

CGCGAATTCGCG was originally synthesized as a substrate for the *Eco*RI restriction endonuclease. Its presence in solutions from which crystals of the endonuclease are being grown leads to a change in crystal form from that of the enzyme alone, fostering the hope that the crystals in the absence of Mg²⁺ contain an abortive binary complex of dodecamer and enzyme suitable for X-ray analysis. If Mg²⁺ is present, the dodecamer is cleaved well by the enzyme (J. Rosenberg, personal communication).

The B helix has been shown here to be the stable conformation of CGCGAATTCGCG in the crystalline state in salt conditions for which CGCG and CGCGCG adopt the left-handed Z configuration. Moreover, the value of 10.1 base pairs per turn and the propeller twist of bases indicate that this crystal structure is close to that which exists in solution. Although the bend in helix axis is probably induced by crystal packing, its presence illustrates the flexibility of the B-DNA structure, and the ease with which the molecule can be continuously deformed to wrap around objects such as the histone core in nucleosomes. Details of the hydration of B-DNA will be of interest as the transitions between B and other forms are generally considered to be a function of water activity². These details, and any sequence-specific modifications of the uniform helix, will be matters of prime concern as refinement continues.

This work was supported by NIH grants GM-12121 and GM-24393, and NSF grant PCM79-13959. H. D. was also the recipient of an NIH Predoctoral Traineeship.

Note added in proof: Further refinement to an R factor of 17.8% has led to a regularization of the O5'-C5'-C4'-C3' backbone torsion angles to a standard *gauche*⁺ (about +60°) conformation.

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Corrigenda

The following acknowledgements should have appeared in the letter 'Endotoxin-induced uveitis in rats as a model for human disease' by J. T. Rosenbaum *et al.* *Nature* **286**, 611–613. 'This work was supported by grant AI 11313 from the NIH. J.T.R. was supported by a fellowship from the Arthritis Foundation.'

In the News and Views article 'X-ray astronomy with the Einstein laboratory' by J. L. Culhane *Nature* **284**, 509–510, reference was made to the 'Harvard Center for Astrophysics'. This should have read 'Harvard/Smithsonian Center for Astrophysics'.

In the News and Views article 'Between the galaxies' by M. G. Edmunds, *Nature* **284**, 213, it was erroneously suggested that the number of intergalactic hydrogen clouds discovered by Sargent *et al.* is considerably *higher* than for galaxies—the correct value was quoted, and the incorrect statement was based on an overestimate of the observed number density of galaxies. Nevertheless, the total mass of the clouds remains small compared with that in galaxies. The author apologises for this error.

In the article 'β-Chain contact sites in the haemoglobin S polymer' by R. L. Nagel *et al.*, *Nature* **283**, 832–834, the credit for Fig. 2 was omitted. Figure 2 was modified from *The Structure and Action of Proteins* by R. E. Dickerson and I. Geis (Benjamin/Cummings, Menlo Park, 1969).

Errata

In the letter 'Oscillation of [Ca²⁺]_i-linked K⁺ conductance in bullfrog sympathetic ganglion cell is sensitive to intracellular anions' by K. Morita *et al.*, *Nature* **283**, 204–205, the column headings for Table 1 should read: 0.5, 1.0 and 3.0 (mM caffeine), not –, 0.5 and 1.0 mM.

In the news item 'Distorting the epidemiology of cancer: the need for a more balanced overview' by R. Peto, *Nature* **284**, 297–300, the caption to the figure on page 299 should have read: Data plotting for 39 countries age-standardized breast cancer death versus total dietary fat (animal or vegetable).

MATTERS ARISING

Leaping dolphins

THE analysis of leaping dolphins by Au and Weihs¹ has some shortcomings. The expenditure of energy during shallow high-speed swimming is presumably correct, although one wonders why a dolphin would not spend at least some time at greater depths if a 4.5-fold decrease in drag can be obtained by going down a metre or so. However, the computation of energy loss during a leaping manoeuvre seems to be at fault. Neglecting spray effects initially, the extra energy required for jumping is given as WH which is the energy required to raise a dolphin of weight W to a height H . However, the energy expended in raising the dolphin against gravity is not lost, but is partially transformed into potential energy at the high point and back to kinetic energy on returning to water-surface level. The dolphin has the same total energy throughout the leap and re-enters the water with the same speed as it had when it emerged. The total loss of energy is zero.

The spray effect, for which an energy loss of mWH is given, has also been computed incorrectly. A dolphin of weight W carries along with it an additional weight of water mW and on leaving the water it leaves this behind in the form of spray. This does not in fact concern the dolphin until it re-enters, when it has to accelerate a similar mass of water up to its swimming speed (U), and so experiences a brief retarding force and a loss of kinetic energy. The loss of energy is clearly $\frac{1}{2}mWU^2/g$, where g is the gravitational constant, and is independent of the angle of the leap. Thus the total energy lost is $\frac{1}{2}mp_d VU^2$, where V and p_d are the volume and density of the dolphin, respectively, and not $\frac{1}{4}(1+m)p_d VU^2$ as the authors claim. They seem to have overestimated the energy loss by a factor of $(1+m)/2m = 3$.

Further, there are more general considerations which could significantly affect the result. It seems unlikely that the dolphin would swim close to the surface between leaps, one reason being the difficulty of launching itself upwards at an angle of 45° from this shallow position. This would require the application of large vertical forces by the fins, of a magnitude several times its own weight. It is more likely that the dolphin follows a sinuous path consisting of leaps out of the water interspersed by dives under the water of a similar shape, thus achieving a clean exit and entry, greatly reducing drag by going deeper while submerged, and presumably breathing while out of the water. It is this total 'flight path' which should be

compared with steady shallow swimming in the no-leap case.

Finally, it is unlikely that a dolphin would leap at an elevation angle as great as 45° because of the resulting loss of speed. A dolphin leaping and diving at this angle reduces his forward speed to about 70% of his speed through the water. Reducing this angle has a small effect on the length of leap, which varies as $\sin 2\alpha$ where α is the elevation angle, but gives a significant improvement in forward speed, which varies approximately as $\cos \alpha$. A better compromise seems to be about 30° , and this is more in accord with my recollections of leaping dolphins.

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1. Au, D. & Weihs, D. *Nature* **284**, 548–550 (1980).

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

AU AND WEIHS' REPLY—Gordon's comments on the calculation of energy consumed during the leap, as well as the manner of leaping, are well taken. We originally considered a model such as his symmetric parabola, but adopted our model because we observed shallow, brief swimming between long leaps after the dolphins shifted to the running mode. Therefore, we treated the problem of comparing the cost of shallow swimming with a jump from and to a shallow depth. The potential energy of the leap would not be useful to the dolphin trying to maintain a shallow swimming depth and must be dissipated in the re-entry splash and turning forces necessary to maintain that depth. Our energy loss J is exactly that required to keep the animal from plunging deeper. We suggested that the shallow

swimming was due to requirements of increased respiration. Gordon's treatment of the overall problem is relevant to the case of sinuous motion whereas our treatment is based on our observations of saltatory motion.

Gordon's approach to calculation of energy loss due to added mass is more accurate than ours. It would result in a 5% increase in the crossover speed U_c at most.

We believe the centre of mass during the leap approximates a parabola with 45° exit angle that maximizes leap length, both because of the great increase in leap length over that occurring during cruising speed and because photographs often suggest a 45° exit. There is often a steep rise out of the water followed by a flattening-over of the body angle once exited that may give the appearance of a lesser exit angle. The difficulty of leaping from, and returning to, a shallow subsurface depth is acknowledged, but we have not seen the kind of motion Gordon postulates. Perhaps the constant extension of the dolphin's pectoral fins during the entire leap, from exit to re-entry, is explained by the manner of locomotion we describe. The pectoral fins probably act as diving planes to redirect the body angle between leaps and this must contribute greatly to the characteristic splashing. We agree that our differences should be resolved with more careful observations and measurements of this difficult-to-observe behaviour.

Finally, we apologize for an error in the last equation of our letter¹ which should read: $U_c^2 = 65.3 V^{1/3}$.

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Climatic warming of the West Antarctic ice sheet

IN our paper on the "Effect of climatic warming on the West Antarctic ice sheet"¹ we included an analysis of the state of equilibrium of the Pine Island and Thwaites glaciers, which drain the northern part of the ice sheet. Our conclusions concerning these glaciers were the result of our own analysis of available data, but we should point out that attention was first drawn to the Pine Island and Thwaites glaciers by Hughes^{2,3}. Moreover, hypothetical Eemian collapse of the West

Antarctic ice sheet⁴ has been under investigation by Hughes, Denton and others as part of the CLIMAP experiments⁵. Results of their analysis were presented during the Symposium on the Dynamics of Large Ice Masses, held in August 1978 at Ottawa; where a review of our work was also presented.

We wish to acknowledge the work of Denton, Hughes and their colleagues concerning the Pine Island and Thwaites glaciers, and we do not claim priority in recognizing the existence of these glaciers, their importance as calving bays, and the possibility that they may currently be "surging".

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Terminal Cretaceous catastrophe

THE statements of Smit and Hertogen¹ that the terminal Cretaceous extinctions were "extremely abrupt" (~200 yr), lacked "warning signal," and that "gradual extinction lose their credibility on more detailed inspection," require substantiation. Simultaneous geological-range terminations of numerous taxa in a stratigraphic section usually indicate a hiatus (missing strata). A terminal Cretaceous marine shallow-water CaCO₃ dissolution event is noted at most marine localities. Was it not operative at Caravaca? Such an event would have caused a terminal Cretaceous hiatus that, in itself, could have accounted for the simultaneous termination of ranges in the Caravaca section, creating the illusion of catastrophic extinctions. Certainly, the termination of ranges via a hiatus would lack a 'warning signal'. Until it is proved that a terminal Cretaceous hiatus does not exist in the Caravaca section, claims of a terminal Cretaceous 'catastrophe' cannot be accepted.

An instantaneous catastrophe phenomenon such as a meteorite impact would have caused synchronous land and marine

extinctions. Smit and Hertogen's claims of "near synchronous extinction" conflicts with Butler *et al.*² and Lindsay *et al.*³ who cast serious doubt on any simultaneity. The statement "the independence of the event from the known normal environmental processes going on in the latest Cretaceous", is unfounded; Smit and Hertogen fail to consider the global warming of deep and shallow marine waters across the Cretaceous-Tertiary boundary noted by Boersma *et al.*⁴ and Margolis *et al.*⁵ They also fail to explain why only planktonic CaCO₃-producing marine organisms were affected significantly by the extinctions. Our Cretaceous-Tertiary dinoflagellate studies along the eastern US—even across a terminal Cretaceous hiatus—record no notable terminal Cretaceous extinctions. Clearly, oceanic pH changes seem to have been a factor in the marine extinctions.

Smit and Hertogen have failed to integrate significant aspects of the geological record into their extraterrestrial extinction model. As such, their use of the term "holocaust" for the terminal Cretaceous extinctions is inappropriate.

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SMIT AND HERTOGEN REPLY—The hiatus assumed by McLean is the least likely explanation for extinctions at the Cretaceous-Tertiary boundary. McLean is confusing the sequence of the thermal events at the boundary and fails to consider the iridium peak, important evidence which we used to postulate a catastrophic (meteoritic) event.

McLean's postulated hiatus would have to be quite large to make it worldwide, and to let it wipe out any geochemical or other 'warning signals'. If such a large hiatus were operative, other taxa like benthic foraminifera and dinoflagellates should reflect a similar "illusion of catastrophic extinction", which is not the case. Further, the expected residual clay deposit from a dissolution event is not present either; a dissolution of the topmost 100 m of the Cretaceous at Caravaca would leave a 20-m thick clay deposit, but only one species would be added to the total of 55 planktonic species that became extinct. We have argued previously^{1,2} that all stratigraphic subdivisions known to be connected with the boundary are present

at Caravaca, including an additional interval, without signs of a hardground or dissolution. McLean needs to prove that a hiatus is present at Caravaca.

While synchronism of extinction is difficult to confirm, the results of Butler *et al.*³ and Lindsay *et al.*⁴ have been seriously questioned^{5–7}. McLean also does not mention a similar magnetostratigraphic investigation by Lerbeckmo *et al.*^{8,9}, who favour synchronism. The problem has not been satisfactorily resolved and a claim of diachronism cannot yet be accepted.

The independence of the event from the normal environmental processes going on in the latest Cretaceous is inherent in the model and it certainly deserves further testing. McLean, however, is confusing cause and effect of the Cretaceous-Tertiary boundary event, when he brings a 'global warming' into the discussion as a normal terminal Cretaceous process. Data of Boersma *et al.*¹⁰ and Margolis *et al.*¹¹ which indicate a warming, are exclusively Palaeocene and postdate the event. Rather a slight cooling is observed from the Cretaceous data only. The 'global warming' of Margolis *et al.*¹¹ indicates that the Cretaceous-Tertiary boundary occurred within a period of high global temperatures, lasting ~40 Myr from mid-Cretaceous to Lower Eocene, but as such there is no reason to relate these to the Cretaceous-Tertiary boundary event.

Lowermost Palaeocene warming¹² may well be a consequence of a large impact; either by direct heat generation or by a sort of 'greenhouse effect' (following oceanic impact) much in the same way as in the model of Alvarez *et al.*¹³. Dinoflagellates may escape the supposed suppression of sunlight by their cyst-forming abilities, as well as the dinoflagellate-like, CaCO₃ producing nannofossils *Braarudosphaera* and *Thoracosphaera*. However, we agree that the biological consequences of a very large impact are largely speculative at the moment.

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BOOK REVIEWS

Hopes and fears for the Islamic world

Abdus Salam

THIS is a remarkable book written by a remarkable young author, not yet 30, addressed to Muslim scholars, intellectuals and scientists. Ziauddin Sardar's basic thesis is that, considering the present state of the Muslim world and of Muslim history of the past four centuries, as well as the urgency of the current global crises, it is imperative that Muslim peoples should think out and work for a planned, rather than an "aimless" future. As the argument develops, its two related dimensions emerge: "to operationalise a living, dynamic civilisation of Islam and to make a positive contribution to the Predicament of Mankind and the stabilisation of Spaceship Earth". In Sardar's view the two tasks are, in essence, one and the same; and both require intellectual boldness and courage.

Sardar starts with a critical look at the recent history of the Muslims. In his view, the Muslim civilization is the only civilization which still preserves something of its essence, and which has the potential to stand up to the dominant civilization of the occident. The tragedy, however, is that all attempts to adapt Western norms and values in the Muslim societies, from the eighteenth century Tanzimat reforms in the Ottoman Empire, to modern strategies of development, have failed to revive their dynamism. "The imitation of the West, has reduced the Muslim intellectuals to the status of voluntary mental slaves. It is now necessary for the Muslims to find solutions to their problems from *within* their own culture and traditional heritage."

In the long run, the author argues, Muslim people can discover their lost vigour and dynamism only by sustained research. He calls for a "Project Umran", an organized research programme to produce detailed conceptual maps and operational plans for alternative Muslim futures. The Muslims have not done their intellectual homework and the first aspect of his research programme calls for a new articulation of the age-old Muslim vision of the Medina State: the city established by the Holy Prophet after his migration from Mecca to Medina. "It is indeed a sorry reflection on the state of Muslim scholarship that almost all accounts of Medina State to date are almost as devoid of freshness and depth as they are of analysis." These are sentiments close to heresy in their boldness so far as traditional scholarship is concerned. And, in this context, Sardar's boldest suggestion is the outline of a modern methodology for the

The Future of Muslim Civilisation. By Ziauddin Sardar. Pp.288. (Croom Helm: 1979.) Hardback £14.95; paperback £4.95. Available from Biblio Distribution Centre, Totowa, New Jersey at \$26.50.

IMAGE
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REASONS

revivification of *ijtihad* (sustained reasoning) which was banned by traditional scholars some 600 years ago. This ban he asserts, was the chief reason for the decline of Muslim civilization. His ultimate picture is of a utopian future Muslim society, with the extended family as its base, the mosque as its focus, devising its own strategies of collective fulfilment, reaffirming commitment to Muslim traditions and values, maintaining elite and leadership accountability through the Islamic concept of criticism and self-criticism (*mahasbaah*), rediscovering the Islamic system of education based on personal contact and community awareness, and synthesizing Western and traditional modes of health care.

Regarding science, Sardar is categorical: "bias-free observation is a myth", he declares. Contemporary science, he argues, is Western science, and only *a* science, and not *the* science.

Nothing in perception is elementary, instantaneous, objective or strictly factual; it is all full of subconscious or indeed preconscious interpretations in the light of previous experience. The emotionally loaded word 'fact' has no place in science; it is a sign of either ignorance or dishonesty on our part if we believe that data gathered

by science are wholly 'pure' and 'external', since they are always intermingled with our world-view and our perceptions [p.208-209].

The Muslims, in his view, must develop their own style of science and rediscover the spirit of science in Islam.

Whether one agrees or disagrees with him — and since I can see no distinction in spirit between, for example, the modern tradition in algebra and that of Omar Khayyam's, or the modern tradition in optics and that of Alhazen's, or Razi's observations on small-pox and their modern extensions, I certainly cannot subscribe to his views so far as *pure* science (as contrasted with goal-orientated technology) is concerned — Sardar's book does mark a sharp departure from conventional Muslim scholarship. He is intellectually demanding of the Muslims, and herein lies the importance of his work. As I indicated above, much of what he has said would spell heresy in some circles (both traditionalist and modernist). By itself, the extensive glossary at the end of the book testifies that his analysis has broken considerable new ground.

But nothing in this brief review can give an adequate idea of the remarkable felicity of Sardar's writing or its urgency and passion. There is his analysis of the "Parameters of Muslim Civilisation", including a historical survey of Muslim intellectual endeavour, an "Anatomy of Muslim Agony", a penetrating analysis of the history of the occidental world-view, and of the present world system.

And then there are the throw-away descriptions — for example of a new breed among Muslim intellectuals, "the professional Muslim" as he is called, whose first characteristic is

... his self-appointed role as a contemporary bishop. The professional Muslim is always at every conference, whatever the theme, whether it is 'Muslim Education' in Mecca, 'Islam and the Challenge of New International Order' in London, 'The Third International Theory' in Tripoli, or 'Muslim Youth' (this despite the fact that he is usually over 45) in Kuala Lumpur.

In the same vein, and even more damning, is Sardar's assessment of the role of Muslim elites in their societies:

... since the day of their independence, the Muslims have been ruled by elite minorities in the name of traditionalism, nationalism, socialism and democracy. These elites have acquired an unchallengeable sense of self-

Mary Evans

importance and omnipotence; they have transformed transient limited goals to universal imperatives. . . . They have sustained a value network characterised by materialism and vulgarity; [putting] blind imitation before critical inquiry, order before justice, obedience before responsibility, elitism and class before humanity, regionalism before universality, packaging before contents, . . . numbers before quality, efficiency before participation, expertise before wisdom, property before people, and economic and political gain before life.

But notwithstanding its power, the book, addressed as it is to the intellectual elites, has its (possibly self-imposed) limitations. There is little assessment of the contemporary Muslim political scene or of the fundamentalist politico-religious or spiritual movements which are currently shaping the Muslim world. It could be that Sardar believes in the transient, limited character of some of the current trends. But whatever these limitations of scope, are we witnessing with this work the emergence of a new, more analytical, impatient and aggressive breed of Muslim scholar? Regrettably, as things are at present in many Muslim countries, such scholars, if they do emerge, will be able to work and write only in the relatively freer atmosphere of the West. □

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Muslim astronomers in the observatory at Istanbul, from a manuscript dated 1571.

The impact of volcanoes through human history

Alexander R. McBirney

Volcanic Activity and Human Ecology. Edited by Payson D. Sheets and Donald K. Grayson. Pp. 644. (Academic: 1980.) \$52, £33.80.

VOLCANISM has recently received a new surge of attention, partly as a result of the eruption of Mount Saint Helens; *Volcanic Activity and Human Ecology* arrives, therefore, at an appropriate time. This book is the first of its kind, so far as I am aware, to attempt a comprehensive treatment of volcanic hazards and their effects on human activity, both modern and ancient. It can be divided into two parts: the first gives a brief summary of the main types of volcanic activity, hazards, tephra and volcanic soils; the second is a series of accounts of specific eruptions and how they affected different cultures.

The descriptions of volcanic activity by Fred Bullard and a chapter on tephra deposits and how they are studied, written by Larry Kittleman, are presented in a simple style easily comprehensible to the non-geologist. A contribution on volcanic

soils by Ugolini and Zasoski provides a great deal of technical data, but would be far less tedious reading had it given less attention to chemical and mineralogical details and more to the practical aspects that interest most readers. Thorarinsson provides a fascinating account of the impact of eruptions in Iceland, and Warrick considers some of the general problems of dealing with volcanic hazards and their social consequences. The techniques of assessing volcanic risks in the Cascade Range are discussed in a paper that was written by Crancell, Mullineaux and Miller shortly before the eruption of Mount Saint Helens, and it makes interesting reading in the light of more recent events. On the whole, the authors fare quite well.

The topics dealt with in the second part of the book were more interesting to me, probably because they were less familiar. I was fascinated by two case studies by Hodge, Sharp and Marts, who describe the effects of recent eruptions on different cultures and economies, one in Hawaii and the other near Mount Baker in the state of Washington. The paper should be required reading for anyone responsible for assess-

ing hazards and dealing with the public under conditions of stress and uncertainty. It shows quite clearly how people with different cultural backgrounds, economic interests and familiarity with a volcano perceive hazards and the restrictions imposed for their safety.

The eruption of Parícutin in Mexico between 1943 and 1952 is treated in considerable depth. Rees describes the impact on the physical environment, and Nolan gives a perceptive and thoughtful account of how five small communities reacted to the disruption of their lives and customs. The ways in which people responded varied widely, depending on the social structures of the communities, and it is particularly interesting to note that those that proved most resilient were people who had strong community ties and were subjected to the least amount of governmental assistance.

Several papers dealing with prehistoric eruptions in western North America address the question of how much importance can be attached to volcanic eruptions as agents affecting the migration, decline or ascendancy of primitive tribes. The contribution by Dumond on Alaska and the Aleutians is particularly notable for its clarity and readable style. The evidence seems to indicate that volcanism only rarely had the

drastic impact on primitive cultures that has been attributed to it. But this is not necessarily true of more advanced societies, such as the classical civilizations of Central America and the Mediterranean. The effects of a huge eruption of Ilopango in El Salvador are shown to have had a profound effect on subsequent patterns of Mayan culture. The degree to which this was true of the eruption of Santorini in 1500 BC is still the subject of controversy. Renfrew discusses evidence for and against the hypothesis that the eruption accounted for the final demise of Minoan civilization, and, using evidence that volcanologists may find less than conclusive, he concludes

that the eruption and the destruction of the community on Crete, dated within 50 years of each other, were coincidental.

No book on the effect of volcanism on civilized communities would be complete without a chapter on the eruption of Vesuvius in AD 79. Jashemski gives us a good summary of the eruption and its effects and explains some of the more recent archaeological work at Pompeii.

It is impossible to enumerate all the interesting facets of this volume. The editors deserve to be complimented on a splendid compilation of well-balanced papers, almost all of which are interesting, timely and well written. One of the most

difficult tasks in a work such as this is keeping the papers on a level that will interest the average reader who is not concerned with details and the pet theories of individual specialists. The editors have managed to do this quite well. In short, the book is an unusually valuable contribution that will be useful to a wide range of readers. □

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A star is born

F.D. Kahn

Star Formation. 10th Advanced Course of the Swiss Society of Astronomy and Astrophysics. By I. Appenzeller, J. L. Lequeux and J. Silk. Pp.222. (Geneva Observatory: Sauverny, Switzerland, 1980.) SwFr.30.

THE brightest stars in spiral galaxies line the insides of the spiral arms. The same regions are rich in interstellar gas, much of it ionized and made luminous by the stars themselves, and concentrations of the dust tend to occur nearby. But the spiral arms are manifestations of a wave-like disturbance that travels at some 100 km s^{-1} relative to the material in the galactic disk. The bright stars must therefore be short-lived, and be born and evolve during the time that the star-forming phase of the wave passes through the region. Stellar structure theory confirms that such stars do indeed consume their nuclear fuel in a relatively short time; the most luminous do so in as little as three million years. All this has been known for two decades or so.

These observations suggest that the diffuse interstellar material provides the source of matter for the new stars, and various theories have been proposed to describe how this might happen. But the sequence of events must be complex: the mean mass density is typically 24 orders of magnitude lower in interstellar space than it is in a star. The condensation process will consequently have various stages, each dominated by a different set of physical parameters. In addition, formidable problems are posed by the angular momentum content of the interstellar gas, and by the magnetic flux linked with it. There must be dissipative processes to reduce them both, otherwise star formation would be completely inhibited.

Many new observations have been made in the past few years which bear on these questions. The spatial and kinematical structure of molecular clouds has been revealed by spectroscopic observations at

millimetre wavelengths. These clouds invariably contain much dust; any stars that form within them are therefore enclosed at birth within a cocoon of dust. The stars thus become initially detectable as infra-red sources, since it is possible only to observe the radiation from the heated dust that surrounds them, and obscures their surfaces. It seems to take some half a million years for this dust to clear away.

The 1980 Advanced Course at Saas Fee was devoted to this range of topics. As always three lecturers took part, and it is clear that this year each one saw his task in a different way. The first set of talks (I. Appenzeller) describes how to use a computer to simulate the process of condensation of matter into a star. The calculations are reasonably tractable if spherical symmetry is assumed. But should the description not be full three-dimensional? Can one even contemplate such a calculation for a system where some important lengths are as large as 10^{17} cm , and others as small as 10^{12} cm ?

In the second group of talks, J. Lequeux sets out to establish some order among the large variety of observational data. He derives empirical formulae to connect the rate of star formation with the local density

of interstellar matter, and to describe the distribution of masses (the initial mass function or IMF) among the newly formed stars. But is there an IMF which applies everywhere? Can the IMF for massive stars be reconciled with that for the lighter stars?

In the last group of talks, J. Silk presents a wide-ranging survey, with no less than 157 references, of recent observational and theoretical results. It is easy to appreciate, after reading his contribution, how complex star formation really is, and how many physical interactions are involved. Yet even so he leaves the impression that an acceptable theory will evolve before long.

The Saas Fee courses have deservedly become famous. The present text maintains their high standard, and provides an excellent guide to a very important subject. The book is well produced and the price most reasonable. But would the organizers be willing to consider a small suggestion? In future, when the three lecturers have spoken, might it not be worthwhile to have another speaker to provide a summary and put the different views in perspective? □

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A summary of 30 years of solar radio astronomy

R. T. Stewart

Introduction to Solar Radio Astronomy and Radio Physics. By A. Kruger. Pp.330. (Reidel: 1979.) Hardback Dfl.95, \$49.95; paperback Dfl.45, \$23.50.

THE last monograph on this subject appeared in 1964, and since then a great number of significant radio observations of the Sun have been made from space and from large ground-based arrays. Considerable progress has also been

achieved in theoretical aspects of plasma emission processes. Thus, the appearance of this volume in which the author attempts to summarize the work carried out during the past 30 years, is timely.

The book is divided into five chapters. In Chapter 1 a short history is given of the early attempts to detect radio emission from the Sun, leading to its discovery in 1942. This is followed by a brief description of the general properties and structure of the Sun. Chapter 2 is devoted to various types of instruments and techniques used in observational solar radio astronomy.

The great variety of radio emissions from the Sun is the subject of the next chapter, which contains many good illustrations. Recent kilometric and hectometric radio observations from spacecraft

of type II and type III bursts are included in this account. Interpretation of these bursts in terms of plasma emission allows the determination of the electron density of the corona from near the Sun to near the Earth. Interpretation of millimetric and centimetric emissions in terms of thermal bremsstrahlung or gyro-synchrotron emission leads to estimates of electron temperatures, electron densities and magnetic field strengths in the chromosphere and low altitudes of the corona. Metric observations cover intermediate altitudes of the corona and give

information on electron density, and the direction and strength of the coronal magnetic field. Hence radio observations cover most of the Sun-Earth environment.

The other important aspect of radio observations is the evidence they provide for non-thermal populations of energetic particles released by solar flares. The fourth chapter deals with the theory of solar radio emission and is divided into primary emission processes, such as thermal bremsstrahlung and gyro-synchrotron emission from accelerated particles, and secondary emission

processes or wave-mode transformations such as Langmuir waves scattering into electromagnetic (plasma) radiation. Plasma instabilities such as the two-stream, velocity-anisotropy and tearing-mode instabilities are briefly discussed. The important question of amplification of radio waves is also mentioned but not considered in detail. The final chapter attempts to integrate solar radio astronomy with solar physics and solar terrestrial physics. The flare phenomenon is discussed, together with several possible mechanisms for the explanation of how particles are accelerated in the short time-scale of the flare and where the flare energy originates.

It is an ambitious undertaking to attempt to cover the entire span of the field in a single volume, but as an extensive review of the scientific literature the book is very successful and contains a useful list of references for the postgraduate student or research scientist. However, as an introduction to solar radio astronomy, it is less successful, being complicated by detail and lacking the highlights which give the reader an overall picture of an area of research. I feel that more emphasis could have been placed on the significant developments — instrumental, observational and theoretical — which have occurred in solar radio astronomy and that this would have led to a more readable account. However, I would recommend this book not only because of its uniqueness but also because it is an honest description of the work done in this field up until 1976. □

R. T. Stewart is a principal research scientist with the Division of Radiophysics of CSIRO, Sydney, Australia, and for the past 16 years has been closely involved with the interpretation of solar radio bursts observed by the Culgoora radioheliograph and other instruments.

Responses to arbitrary time schedules

J. T. Enright

Time in Animal Behaviour. By M. Richelle and H. Lejeune. Pp.273. (Pergamon: 1980.) Hardback £22, \$51; flexi £9.50, \$23.

DO NOT be misled by the title, the jacket and the forward of this book. It does not treat general features of behavioural adjustment to time, but instead deals almost exclusively with the key- and lever-pressing performances of hungry rats, pigeons and a small number of other species. The introductory chapter led me to hope for an integration of biological-clock research (circadian and similar studies) with other broader aspects of temporal regulation, but, as one finally discovers on p.135: "In the almost total absence of [relevant biological-clock] experimental work, we shall content ourselves with defining the type of research that is strongly needed to fill the gap. . .".

The real intent of the authors is to introduce certain sorts of temporally programmed operant-conditioning experiments to the uninitiated. These experiments centre around one of two basic designs. On the "fixed interval schedule", the animal learns not to "emit responses" (I shudder) continually or randomly, but to wait, after a given success, for a major fraction of the imposed unrewarded interval before trying again, and then to try persistently until success finally comes. At the other extreme is "differential reinforcement of low rates", a programme in which any unsuccessful keypress resets the timer to zero; following a reward, the animal is required to wait for *at least* the pre-programmed interval before trying again. Granted proper shaping regimes, most animals can apparently master these schedules, as well as many complex variants. True enough, mastery of the programme usually involves only relatively imprecise indications that the animal has some sort of expectation in the time domain. Nevertheless, the evidence presented in this book clearly demonstrates

that animals can evaluate and respond to arbitrarily chosen time intervals ranging from a few seconds to a few minutes.

The arbitrariness of the selected time intervals, based on their convenience in an operant-conditioning context, deserves emphasis. Most biologists are already familiar with certain other animal capacities to utilize time as a variable in an ecological context — matters of microseconds, as when an insectivorous bat uses sonar to home in on its prey, up to intervals of days, in time-training (*Zeitgedächtnis*) experiments with honeybees, and even weeks, in semi-lunar reproductive rhythms. Such aspects of temporal adaptation are presumably the intensely selected consequences of prolonged evolution. The animal skills considered in this book, on the other hand, seem to be completely devoid of any ecological relevance. To me, the impressive aspect of the results is that the animals show any capacity at all to deal appropriately with the arbitrary time schedules provided. These abilities are probably an evolutionary by-product, but the natural context in which they might originate remains obscure.

The general audience, for whom the book is intended, will encounter a number of stumbling blocks. It is easy to lose sight of the central questions when confronted with detailed results from yet another minor variant on the small number of standard experimental designs. A further difficulty lies in the authors' propensity for abbreviations which, once defined, the reader is expected to remember throughout subsequent chapters. The pages are peppered with FI, FR, FT, FI-FR, DRL 20s, DRL 60 LH 6, IRT, TO, RF/R and FRC, on through MULT FR 25 FI 7 min.

The authors emphasize (Chapter 6) that although brain lesions and certain drugs affect the response patterns, such results are fraught with ambiguity. I myself find the authors' attempts at overall interpretation (Chapter 9) to be equally ambiguous. For example, "We are dealing

here with a relaxation phenomenon, and not a pendulum-type oscillatory system. . . Temporal regulation involves the buildup of some sort of tension that is suddenly discharged. . ." (p.222). That sort of conclusion seems to me to be inextricably tied up with the experimental designs; if the imposed timing schedule contains major elements of a relaxation oscillation (sudden reset to zero), how could the animals behave otherwise? What we have here, then, is a large body of experimental data which seem, at present, to be begging for adequate explanation at both the evolutionary and the neurophysiological level. The authors deserve our thanks for making these results accessible in a compact form for those — like myself — who have been unfamiliar with this body of empirical knowledge. □

J. T. Enright is Professor of Behavioral Physiology at the Scripps Institution of Oceanography, La Jolla, California.

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